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<b>(21) International Application Number:</b> PCT/US93/07189 <b>(22) International Filing Date:</b> 29 July 1993 (29.07.93)  <b>(30) Priority data:</b> 923,780 31 July 1992 (31.07.92) US 029,335 4 March 1993 (04.03.93) US 040,510 31 March 1993 (31.03.93) US  <b>(71) Applicant:</b> CREATIVE BIOMOLECULES, INC. [US/ US]; 45 South Street, Hopkinton, MA 01748 (US).  <b>(72) Inventors:</b> JONES, William, K. ; 35 Saint Paul Street, Brookline, MA 02116 (US). TUCKER, Ronald, F. ; 132 Robert Road, Holliston, MA 01746 (US). RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). OPPERMAN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). OZKAYNAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). KUBERA- SAMPATH, Thangavel ; Six Spring Street, Medway, MA 02053 (US).		<b>(74) Agent:</b> KELLEY, Robin, D.; Testa, Hurwitz & Thibault, Exchange Place, 53 State Street, Boston, MA 02109 (US).  <b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>	
<b>(54) Title:</b> MORPHOGENIC PROTEIN SOLUBLE COMPLEX AND COMPOSITION THEREOF			
<b>(57) Abstract</b>  Disclosed are compositions of morphogenic proteins constituting soluble forms of these proteins, antibodies that distinguish between soluble and mature forms, and method for producing these morphogenic proteins and antibodies.			

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MORPHOGENIC PROTEIN SOLUBLE COMPLEX AND COMPOSITION THEREOF.Field of the Invention

The present invention relates generally to  
5 morphogenic proteins and, more particularly, to  
compositions having improved solubility in aqueous  
solvents.

Background of the Invention

10 Morphogenic proteins ("morphogens") are well known  
and described in the art. See, for example, U.S. Pat.  
Nos. 4, 968,590; 5,011,691; 5,018,753; PCT US92/01968 and  
PCT US92/07432; as well as various articles published in  
the scientific literature, including Ozkaynak et al.  
15 (1992) J.Biol. Chem. 267:25220-25227 and Ozkaynak et al.  
(1991) Biochem. Biophys. Res. Comm. 179:116-123. The  
art has described how to isolate morphogenic proteins  
from bone, how to identify genes encoding these proteins  
and how to express them using recombinant DNA technology.  
20 The morphogenic proteins are capable of inducing  
endochondral bone formation and other tissue formation in  
a mammal when they are properly folded, dimerized and  
disulfide bonded to produce a dimeric species having the  
appropriate three dimensional conformation. The proteins  
25 have utility in therapeutic applications, either by  
direct or systemic administration. Where bone induction  
is desired, for example, the morphogen typically is  
provided to the desired site for bone formation in a  
mammal in association with a suitable matrix having the  
30 appropriate conformation to allow the infiltration,  
proliferation and differentiation of migrating progenitor  
cells. The morphogenic protein adsorbed to the surfaces

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of a suitable matrix is generally referred to in the art as an osteogenic device. The proteins can be isolated from bone or, preferably, the gene encoding the protein is produced recombinantly in a suitable host cell.

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The morphogen precursor polypeptide chains share a common structural motif, including a N-terminal signal sequence and pro region, both of which are cleaved to produce a mature sequence, capable of disulfide bonding and comprising an N-terminal extension and a C-terminal domain whose amino acid sequence is highly conserved among members of the family. In their mature dimeric forms, the morphogens typically are fairly insoluble under physiological conditions. Increasing the solubility of these proteins has significant medical utility as it would enhance systemic administration of morphogens as therapeutics. Various carrier proteins, including serum albumin and casein are known to increase the solubility of morphogens (see, for example, PCT US92/07432). PCT US92/05309 (WO 93/00050) discusses the use of various solubilizing agents, including various amino acids and methyl esters thereof, as well as guanidine, sodium chloride and heparin, to increase the solubility of mature dimeric BMP2.

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Improved methods for the recombinant expression of morphogenic proteins is an ongoing effort in the art. It is an object of this invention to provide an improvement in the methods for producing and purifying morphogenic proteins having high specific activity, and for formulating compositions and osteogenic devices comprising these proteins. Another object is to provide soluble forms of morphogenic proteins consisting essentially of amino acid sequences derived from

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morphogenic proteins. Another object is to provide formulations which stabilize the soluble complex of morphogenic proteins. Still another object is to provide means for distinguishing between soluble forms of the  
5 protein and the mature morphogenic species, to provide means for quantitating the amounts of these proteins in a fluid, including a body fluid, such as serum, cerebro-sprinal fluid or peritoneal fluid, and to provide polyclonal and monoclonal antibodies capable of  
10 distinguishing between these various species.

Another object is to provide antibodies and biological diagnostic assays for monitoring the concentration of morphogens and endogenous anti-morphogen  
15 antibodies present in a body fluid and to provide kits and assays for detecting fluctuations in the concentrations of these proteins in a body fluid. U.S. Patent No. 4,857,456 and Urist et al. (1984) Proc. Soc. Exp. Biol. Med. 176:472-475 describe a serum assay for  
20 detecting a protein purported to be a bone morphogenetic protein. The protein is not a member of the morphogen family of proteins described herein, differing in molecular weight, structural characteristics and solubility from these proteins.

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#### Summary of the Invention

It now has been discovered that morphogenic protein secreted into cultured medium from mammalian cells contains as a significant fraction of the secreted  
30 protein a soluble form of the protein, and that this soluble form comprises the mature dimeric species, including truncated forms thereof, noncovalently associated with at least one, and preferably two pro domains. It further has been discovered that antibodies

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can be used to discriminate between these two forms of the protein. These antibodies may be used as part of a purification scheme to selectively isolate the mature or the soluble form of morphogenic protein, as well as to

5   quantitate the amount of mature and soluble forms produced. These antibodies also may be used as part of diagnostic treatments to monitor the concentration of morphogenic proteins in solution in a body and to detect

10   fluctuations in the concentration of the proteins in their various forms. The antibodies and proteins also may be used in diagnostic assays to detect and monitor concentrations of endogenous anti-morphogen antibodies to the various forms of these proteins in the body.

15       An important embodiment of the invention is a dimeric protein comprising a pair of polypeptide subunits associated to define a dimeric structure having morphogenic activity. As defined herein and in parent, related applications, morphogens generally are capable

20   of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the

25   growth and maintenance of differentiated cells.

Each of the subunits of the dimeric morphogenic protein comprises at least the 100 amino acid peptide sequence having the pattern of seven or more cysteine

30   residues characteristic of the morphogen family. Preferably, at least one of the subunits comprises the mature form of a subunit of a member of the morphogen family, or an allelic, species, chimeric or other sequence variant thereof, noncovalently complexed with a

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peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species, chimeric or other sequence variant thereof. The pair of subunits and one or, preferably, two pro region peptides, together form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.

Preferably, both subunits comprise a mature form of a subunit of a member of the morphogen family or an allelic, species, chimeric or other sequence variant thereof, and both subunits are noncovalently complexed with a peptide comprising a pro region, or a fragment thereof. Most preferably, each subunit is the mature form of human OP-1, or a species, allelic or other sequence variant thereof, and the pro region peptide is the entire or partial sequence of the pro region of human OP-1, or a species, allelic, chimeric or other sequence variant thereof. Currently, preferred pro regions are full length forms of the pro region. Pro region fragments preferably include the first 18 amino acids of the pro sequence. Other useful pro region fragments are truncated sequences of the intact pro region sequence, the truncation occurring at the proteolytic cleavage site Arg-Xaa-Xaa-Arg. As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

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As used herein, the mature form of a morphogen protein subunit includes the intact C-terminal domain and intact or truncated forms of the N-terminal extensions. For example, useful mature forms of OP-1 include dimeric species defined by residues 293-431 of Seq ID No. 1, as well as truncated sequences thereof, including sequences defined by residues 300-431, 313-431, 315-431, 316-431 and 318-431. Note that this last sequence retains only about the last 10 residues of the N-terminal extension sequence. Fig. 2 presents the N-terminal extensions for a number of preferred morphogen sequences. Canonical Arg-Xaa-Xaa-Arg cleavage sites where truncation may occur are boxed or underlined in the figure. As will be appreciated by those having ordinary skill in the art, mature dimeric species may include subunit combinations having different N-terminal truncations.

Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins (see below), as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain.

The soluble proteins of this invention also are useful in the formation of therapeutic compositions for administration to a mammal, particularly a human, and for the development of biological assays for monitoring the concentration of these proteins and endogenous antibodies to these proteins in cell samples and body fluids, including, but not limited to, serum, cerebrospinal fluid and peritoneal fluid.

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The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of the invention.

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#### Brief Description of the Drawings

Fig. 1 is a schematic representation of a morphogen polypeptide chain as expressed from a nucleic acid encoding the sequence, wherein the cross-hatched region represents the signal sequence; the stippled region represents the pro domain; the hatched region represents the N-terminus ("N-terminal extension") of the mature protein sequence; and the open region represents the C-terminal region of the mature protein sequence defining the conserved seven cysteine domain, the conserved cysteines being indicated by vertical hatched lines;

Fig.2 lists the sequences of the N-terminal extensions of the mature forms of various morphogens; and

Fig. 3 is a gel filtration column elution profile of a soluble morphogen (OP-1) produced and purified from a mammalian cell culture by IMAC, S-Sepharose and S-200HR chromatography in TBS (Tris-buffered saline), wherein  $V_0$  is the void volume, ADH is alcohol dehydrogenase (MW 150 kDa), BSA is bovine serum albumin (MW 67 kDa), CA is carbonic anhydrase (MW 29kDa) and CytC is cytochrome C (MW 12.5 kDa).

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### Detailed Description

A soluble form of morphogenic proteins now has been discovered wherein the proteins consist essentially of the amino acid sequence of the protein. The soluble form is a non-covalently associated complex comprising the pro domain or a fragment thereof, noncovalently associated or complexed with a dimeric protein species having morphogenic activity, each polypeptide of the dimer having less than 200 amino acids and comprising at least the C-terminal six, and preferably seven cysteine skeleton defined by residues 330-431 and 335-431, respectively, of Seq. ID No. 1. Preferably, the polypeptide chains of the dimeric species comprise the mature forms of these sequences, or truncated forms thereof. Preferred truncated forms comprise the intact C-terminal domain and at least 10 amino acids of the N-terminal extension sequence. The soluble forms of these morphogenic proteins may be isolated from cultured cell medium, a mammalian body fluid, or may be formulated in vitro.

In vivo, under physiological conditions, the pro domain may serve to enhance the transportability of the proteins, and/or to protect the proteins from proteases and scavenger molecules, including antibodies. The pro domains also may aid in targeting the proteins to a particular tissue and/or to present the morphogen to a morphogen cell surface receptor by interaction with a co-receptor molecule. The isolated proteins may be used

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in therapeutic formulations, particularly for oral or parenteral administration, and in the development of diagnostic and other tissue evaluating kits and assays to monitor the level of endogenous morphogens and endogenous  
5 anti-morphogen antibodies.

Detailed descriptions of the utility of these morphogens in therapies to regenerate lost or damaged tissues and/or to inhibit the tissue destructive  
10 effects of tissue disorders or diseases, are provided in international applications US92/01968 (WO92/15323); US92/07358 (WO93/04692) and US92/07432 (WO93/05751) the disclosures of which are incorporated herein by reference. Morphogens, including the soluble morphogen  
15 complexes of this invention, are envisioned to have particular utility as part of therapies for regenerating lost or damaged bone, dentin, periodontal, liver, cardiac, lung and nerve tissue, as well as for protecting these tissues from the tissue destructive  
20 effects associated with an immunological response. The proteins also are anticipated to provide a tissue protective effect in the treatment of metabolic bone disorders, such as osteoporosis, osteomalacia and osteosarcoma; in the treatment of liver disorders,  
25 including cirrhosis, hepatitis, alcohol liver disease and hepatic encephalopathy; and in the treatment or prevention of ischemia reperfusion-associated tissue damage, particularly to nerve or cardiac tissue.

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Presented below are detailed descriptions of useful soluble morphogen complexes of this invention, as well as how to make and use them.

5 I. Useful Soluble Morphogen Complexes - Protein Considerations

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins,  
10 such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), 60A protein (from  
15 *Drosophila*, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218), and the recently identified OP-3.

The members of this family, which are a subclass of the TGF- $\beta$  super-family of proteins, share characteristic  
20 structural features, represented schematically in Fig. 1, as well as substantial amino acid sequence homology in their C-terminal domains, including a conserved seven cysteine structure. As illustrated in the figure, the proteins are translated as a precursor polypeptide  
25 sequence 10, having an N-terminal signal peptide sequence 12, (the "pre pro" region, indicated in the figure by cross-hatching), typically less than about 30 residues, followed by a "pro" region 14, indicated in the figure by stippling, and which is cleaved to yield the mature  
30 sequence 16. The mature sequence comprises both the conserved C-terminal seven cysteine domain 20, and an N-terminal sequence 18, referred to herein as an N-terminal extension, and which varies significantly in sequence between the various morphogens. Cysteines are

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represented in the figure by vertical hatched lines 22.  
The polypeptide chains dimerize and these dimers  
typically are stabilized by at least one interchain  
disulfide bond linking the two polypeptide chain  
5 subunits.

The signal peptide is cleaved rapidly upon  
translation, at a cleavage site that can be predicted in  
a given sequence using the method of Von Heijne ((1986)  
10 Nucleic Acids Research 14:4683-4691.) The "pro" form of  
the protein subunit, 24, in Fig. 1, includes both the pro  
domain and the mature domain, peptide bonded together.  
Typically, this pro form is cleaved while the protein is  
still within the cell, and the pro domain remains  
15 noncovalently associated with the mature form of the  
subunit to form a soluble species that appears to be the  
primary form secreted from cultured mammalian cells.  
Typically, previous purification techniques utilized  
denaturing conditions that disassociated the complex.

20 Other soluble forms of morphogens secreted from  
mammalian cells include dimers of the pro forms of these  
proteins, wherein the pro region is not cleaved from the  
mature domain, and "hemi-dimers", wherein one subunit  
25 comprises a pro form of the polypeptide chain subunit and  
the other subunit comprises the cleaved mature form of  
the polypeptide chain subunit (including truncated forms  
thereof), preferably noncovalently associated with a  
cleaved pro domain.

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The isolated pro domain typically has a substantial  
hydrophobic character, as determined both by analysis of  
the sequence and by characterization of its properties in  
solution. The isolated pro regions alone typically are

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not significantly soluble in aqueous solutions, and require the presence of denaturants, e.g., detergents, urea, guanidine HCl, and the like, and/or one or more carrier proteins. Accordingly, without being limited to  
5 any given theory, the non-covalent association of the cleaved pro region with the mature morphogen dimeric species likely involves interaction of a hydrophobic portion of the pro region with a corresponding hydrophobic region on the dimeric species, the  
10 interaction of which effectively protects or "hides" an otherwise exposed hydrophobic region of the mature dimer from exposure to aqueous environments, enhancing the affinity of the mature dimer species for aqueous solutions.

15 Morphogens comprise a subfamily of proteins within the TGF- $\beta$  superfamily of structurally related proteins. Like the morphogens described herein, TGF- $\beta$  also has a pro region which associates non-covalently with the  
20 mature TGF- $\beta$  protein form. However, unlike the morphogens, the TGF- $\beta$  pro region contains numerous cysteines and forms disulfide bonds with a specific binding protein. The TGF- $\beta$ 1 pro domain also is phosphorylated at one or more mannose residues, while the  
25 morphogen pro regions typically are not.

Useful pro domains include the full length pro regions described below, as well as various truncated forms hereof, particularly truncated forms cleaved at  
30 proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region comprises the full length form rather than

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a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen (see below). Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion of one or more morphogen pro sequences.

Table I, below, describes the various preferred morphogens identified to date, including their nomenclature as used herein, the sequences defining the various regions of the subunit sequences, their Seq. ID references, and publication sources for their nucleic acid and amino acid sequences. The disclosure of these publications is incorporated herein by reference. The mature protein sequences defined are the longest anticipated forms of these sequences. As described above, shorter, truncated forms of these sequences also are contemplated. Preferably, truncated mature sequences include at least 10 amino acids of the N-terminal extension. Fig. 2 lists the N-terminal extensions for a number of the preferred morphogen sequences described below. Arg-Xaa-Xaa-Arg cleavage sites that may yield truncated sequences of the mature subunit form are boxed or underlined in the figure.

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TABLE I

	"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1"), or mouse OP-1 ("mOP-1".) The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 1 and 2 (hOP1) and Seq. ID Nos. 3 and 4 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1), wherein the conserved seven cysteine skeleton is defined by residues 330-431 and 329-430, respectively, and the N-terminal extensions are defined by residues 293-329 and 292-329, respectively. The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins, are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).
5		
10		
15		
20		
25		
30	"OP-2"	refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2") or mouse OP-2 ("mOP-2".) The full length proteins are provided in Seq. ID Nos. 5 and 6 (hOP2) and Seq. ID Nos. 7 and 8 (mOP2.) The mature proteins are defined

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essentially by residues 264-402 (hOP2) and 261-399 (mOP2), wherein the conserved seven cysteine skeleton is defined by residues 301-402 and 298-399, respectively, and the N-terminal extensions are defined by residues 264-300 and 261-297, respectively. The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

"OP-3" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-3 protein, including allelic and species variants thereof, e.g., mouse OP-3 ("mOP-3".) The full length protein is provided in Seq. ID No. 9. The mature protein is defined essentially by residues 261-399 or 264-399, wherein the conserved seven cysteine skeleton is defined by residues 298-399 and the N-terminal extension is defined by residues 264-297 or 261-297. The "pro" region of the protein, cleaved to yield the mature, morphogenically active proteins likely is defined essentially by residues 20-262.

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"BMP2/BMP4" refers to protein sequences encoded by the human BMP2 and BMP4 genes. The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Seq. ID Nos. 10 and 11, respectively, and in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396, of which residues 249-296/283-296 define the N-terminal extension and 295-396 define the C-terminal domain. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408, of which 257-307/293-307 define the N-terminal extension, and 308-408 define the C-terminal domain.

"DPP" refers to protein sequences encoded by the Drosophila DPP gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 12 and in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588, where residues 457-586 define the N-terminal extension and 487-588 define the C-terminal domain.

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- "Vgl" refers to protein sequences encoded by the Xenopus Vgl gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 13 and in Weeks (1987) Cell 51: 861-867. The pro domain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360, where residues 247-258 define the N-terminal extension, and residues 259-360 define the C-terminal domain.
- "Vgr-1" refers to protein sequences encoded by the murine Vgr-1 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq. ID No. 14 and in Lyons, et al, (1989) PNAS 86: 4554-4558. The pro domain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438, where residues 300-336 define the N-terminal extension and residues 337-438 define the C-terminus.
- "GDF-1" refers to protein sequences encoded by the human GDF-1 gene. The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 15 and Lee (1991) PNAS 88:4250-4254. The pro domain

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likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372, where residues 215-256 define the N-terminal extension and residues 257-372 define the C-terminus.

"60A" refers to protein sequences encoded by the *Drosophila* 60A gene. The amino acid sequence for the full length protein appears in Seq. ID No. 16 and in Wharton et al. (1991) PNAS 88:9214-9218) The pro domain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455, wherein residues 325-353 define the N-terminal extension and residues 354-455 define the C-terminus.

"BMP3" refers to protein sequences encoded by the human BMP3 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 17 and in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472, wherein residues 291-370 define the N-terminal extension and residues 371-472 define the C-terminus.

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- "BMP5" refers to protein sequences encoded by the human BMP5 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 18 and in Celeste, et al. (1990) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454, where residues 317-352 define the N-terminus and residues 352-454 define the C-terminus.
- "BMP6" refers to protein sequences encoded by the human BMP6 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq. ID No. 16 and in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513, where residues 375-411 define the N-terminus and residues 412-513 define the C-terminus.

Note that the OP-2 and OP-3 proteins have an additional cysteine residue in the C-terminal region (e.g., see residue 338 in these sequences), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton

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("Gly-Gly-Pro-Pro") but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine  
5 skeleton.

The dimeric morphogen species are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this  
10 invention. Thus, as defined herein, a morphogen useful in a soluble morphogen complex is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain has less than 200 amino acids and comprises at least the C-terminal six, preferably seven  
15 cysteine skeleton defined by residues 335-431 of Seq. ID No. 1, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not  
20 their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain  
25 disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. The solubility of these structures is improved when the mature dimeric form of a morphogen, in accordance with the invention, is complexed with at least one, and  
30 preferably two, pro domains.

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Various generic sequences (Generic Sequence 1-6) defining preferred C-terminal sequences useful in the soluble morphogens of this invention are described in USSN 07/923,780, incorporated herein above by  
5 reference. Two currently preferred generic sequences are described below.

Generic Sequence 7 (Seq. ID No. 20) and Generic Sequence 8 (Seq. ID No. 21) disclosed below,  
10 accommodate the homologies shared among preferred morphogen protein family members identified to date, including OP-1, OP-2, OP-3, CBMP2A, CBMP2B, BMP3, 60A, DPP, Vgl, BMP5, BMP6, Vrg-1, and GDF-1. The amino acid sequences for these proteins are described herein (see  
15 Sequence Listing) and/or in the art, as well as in PCT publication US 92/07358, (WO93/04692), for example. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons  
20 (Generic Sequences 7 and 8, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences allow for an additional cysteine at position 41 (Generic Sequence 7) or position 46 (Generic Sequence 8), providing an  
25 appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

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Generic Sequence 7

```

          Leu Xaa Xaa Xaa Phe
              1               5
5      Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa
              10
          Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
              15               20
          Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
10              25               30
          Xaa Pro Xaa Xaa Xaa Xaa Xaa
              35
          Xaa Xaa Xaa Asn His Ala Xaa Xaa
              40               45
15      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              50
          Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
              55               60
          Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
20              65
          Xaa Xaa Xaa Leu Xaa Xaa Xaa
              70               75
          Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
              80
25      Xaa Xaa Xaa Xaa Met Xaa Val Xaa
              85               90
          Xaa Cys Xaa Cys Xaa
              95

```

wherein each Xaa is independently selected from a group  
 30 of one or more specified amino acids defined as  
 follows: "Res." means "residue" and Xaa at res.2 =  
 (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4  
 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys  
 or Ala); Xaa at res.7 = (Asp or Glu); Xaa at res.8 =

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(Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala  
5 or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln, Ala or Ser);  
10 Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa  
15 at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu, Met or Ile); Xaa at  
20 res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at  
25 res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val, Pro or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or  
30 Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro, Val or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at

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res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Leu, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res.86 = (Tyr, Glu or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu, Trp or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp, Gln or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

As described above, Generic Sequence 8 (Seq. ID No. 21) includes all of Generic Sequence 7 and in addition includes the following sequence at its N-terminus:

Cys Xaa Xaa Xaa Xaa  
1 5

25 Accordingly, beginning with residue 7, each "Xaa"  
in Generic Seq. 8 is a specified amino acid defined as  
for Generic Seq. 7, with the distinction that each  
residue number described for Generic Sequence 7 is  
shifted by five in Generic Seq. 8. Thus, "Xaa at res.2  
30 =(Tyr or Lys)" in Gen. Seq. 7 refers to Xaa at res. 7

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in Generic Seq. 8. In Generic Seq. 8, Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); and Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).

5  
Accordingly, other useful sequences defining preferred C-terminal sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of  
10 the sequences incorporated into Generic Seq. 7 and 8 above. These are anticipated to include allelic, species, chimeric and other sequence variants, (e.g., including "muteins" or "mutant proteins"), whether naturally-occurring or biosynthetically produced, as  
15 well as novel members of this morphogenic family of proteins. As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are  
20 conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with  
25 a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino  
30 acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two

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aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNASTAR, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

Also as used herein, "sequence variant" is understood to mean an amino acid sequence variant form of the morphogen protein, wherein the amino acid change or changes in the sequence do not alter significantly the morphogenic activity (e.g., tissue regeneration activity) of the protein, and the variant molecule performs substantially the same function in substantially the same way as the naturally-occurring form of the molecule. Sequence variants may include single or multiple amino acid changes, and are intended to include chimeric sequences as described below. The variants may be naturally-occurring or may be biosynthetically induced by using standard recombinant DNA techniques or chemical protein synthesis methodologies.

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The currently most preferred protein sequences useful in soluble morphogen complexes in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 335-431 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein.

Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 22).

In still another preferred aspect of the invention, useful morphogens include active proteins comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding the conserved C-terminal cysteine domain of OP1 or OP2, e.g., defined by nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. ID Nos. 1 and 5, respectively, under stringent hybridization conditions. As used herein, stringent hybridization conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

Similarly, in another preferred aspect of the invention, useful pro region peptides include polypeptide chains comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding at least the N-terminal 18 amino

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acids of the pro region sequences for any of the sequences listed in Seq. ID Nos. 1-19, under stringent hybridization conditions. Most preferably, the peptides are encoded by nucleic acids that hybridize to  
5 the DNA or RNA sequences encoding at least the N-terminal 18 amino acids of the pro region sequences for OP1 or OP2, e.g., nucleotides 136-192 and nucleotides 152-211 of Seq. ID Nos. 1 and 5, respectively.

10

Useful N-terminal extension sequences are listed in Fig. 2 for use with the C-terminal domains described above. Also as described above, the full length N-terminal extensions, or truncated forms thereof, may be  
15 used in preferred dimeric species. The mature dimeric species may be produced from intact DNAs, or truncated forms thereof. It also is envisioned as an embodiment of the invention that chimeric morphogen sequences can be used. Thus, DNAs encoding chimeric morphogens may  
20 be constructed using part or all of the N-terminal extension from one morphogen and a C-terminal domain derived from one or more other morphogens. These chimeric proteins may be synthesized using standard recombinant DNA methodology and/or automated chemical  
25 nucleic acid synthesis methodology well described in the art. Other chimeric morphogens include soluble morphogen complexes where the pro domain is encoded from a DNA sequence corresponding to one or more morphogen pro sequences, and part or all of the mature  
30 domain is encoded by DNA derived from one or more

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other, different morphogens. These soluble chimerics may be produced from a single synthetic DNA as described below, or, alternatively, may be formulated in vitro from isolated components also as described  
5 herein below.

Finally, the morphogen pro domains and/or mature form N-terminal extensions themselves may be useful as tissue targeting sequences. As described above, the  
10 morphogen family members share significant sequence homology in their C-terminal active domains. By contrast, the sequences diverge significantly in the sequences which define the pro domain and the N-terminal 39 amino acids of the mature protein.  
15 Accordingly, the pro domain and/or N-terminal extension sequence may be morphogen-specific. Accordingly, part or all of these morphogen-specific sequences may serve as tissue targeting sequences for the morphogens described herein. For example, the N-terminal  
20 extension and/or pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Thus, for example, the morphogen-specific sequences of OP-1, BMP2 or BMP4, all of which proteins  
25 are found naturally associated with bone tissue (see, for example, US Pat. No. 5,011,691) may be particularly useful sequences when the morphogen complex is to be targeted to bone. Similarly, BMP6 (or Vgr-1) specific sequences may be used when targeting to lung tissue is  
30 desired. Alternatively, the morphogen-specific sequences of GDF-1 may be used to target soluble

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morphogen complexes to nerve tissue, particularly brain tissue, where GDF-1 appears to be primarily expressed (see, for example, Lee, PNAS, 88:4250-4254 (1991), incorporated herein by reference).

5

## II. Recombinant Production of Soluble Morphogen Complexes

- Soluble morphogen complexes can be produced from  
10 eukaryotic host cells, preferably mammalian cells, using standard recombinant expression techniques. An exemplary protocol currently preferred, is provided below, using a particular vector construct and chinese hamster ovary (CHO) cell line. Those skilled in the  
15 art will appreciate that other expression systems are contemplated to be useful, including other vectors and other cell systems, and the invention is not intended to be limited to soluble morphogenic protein complexes produced only by the method detailed hereinbelow.
- 20 Similar results to those described herein have been observed using recombinant expression systems developed for COS and BSC cells.

- Morphogen DNA encoding the precursor sequence is  
25 subcloned into an insertion site of a suitable, commercially available pUC-type vector (e.g., pUC-19, ATCC #37254, Rockville, MD), along with a suitable promoter/enhancer sequences and 3' termination sequences. Useful DNA sequences include the published  
30 sequences encoding these proteins, and/or synthetic constructs. Currently preferred promoter/enhancer sequences are the CMV promoter (human cytomegalovirus major intermediate - early promoter) and the mouse

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mammary tumor virus promoter (mMTV) boosted by the rous sarcoma virus LTR enhancer sequence (e.g., from Clontech, Inc., Palo Alto). Expression also may be further enhanced using transactivating enhancer  
5 sequences. The plasmid also contains DHFR as an amplifiable marker, under SV40 early promoter control (ATCC #37148). Transfection, cell culturing, gene amplification and protein expression conditions are standard conditions, well known in the art, such as are  
10 described, for example in Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989). Briefly, transfected cells are cultured in medium containing 0.1-0.5% dialyzed fetal calf serum (FCS) and stably transfected high expression cell lines  
15 are obtained by subcloning and evaluated by standard Western or Northern blot. Southern blots also are used to assess the state of integrated sequences and the extent of their copy number amplification.

20 A currently preferred expression vector contains the DHFR gene, under SV40 early promoter control, as both a selection marker and as an inducible gene amplifier. The DNA sequence for DHFR is well characterized in the art, and is available  
25 commercially. For example, a suitable vector may be generated from pMAM-neo (Clontech, Inc., Palo Alto, CA) by replacing the neo gene (BamHI digest) with an SphI-BamHI, or a PvuII-BamHI fragment from pSV5-DHFR (ATCC #37148), which contains the DHFR gene under SV40 early  
30 promoter control. A BamHI site can be engineered at the SphI or PvuII site using standard techniques (e.g., by linker insertion or site-directed mutagenesis) to allow insertion of the fragment into the vector backbone. The morphogen DNA can be inserted into the

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polylinker site downstream of the MMTV-LTR sequence (mouse mammary tumor virus LTR). The CMV promoter sequence then may be inserted into the expression vector (e.g., from pCDM8, Invitrogen, Inc.) The SV40  
5 early promoter, which drives DHFR expression, preferably is modified in these vectors to reduce the level of DHFR mRNA produced.

The currently preferred mammalian cell line is a  
10 CHO Chinese hamster ovary, cell line, and the preferred procedure for establishing a stable morphogen production cell line with high expression levels comprises transfecting a stable CHO cell line, preferably CHO-DXB11, with the expression vector  
15 described above, isolating clones with high morphogen expression levels, and subjecting these clones to cycles of subcloning using a limited dilution method described below to obtain a population of high expression clones. Subcloning preferably is performed  
20 in the absence of MTX to identify stable high expression clones which do not require addition of MTX to the growth media for morphogen production.

In the subcloning protocol cells are seeded on ten  
25 100mm petri dishes at a cell density of either 50 or 100 cells per plate, with or preferably without MTX in the culture media. After 14 days of growth, clones are isolated using cloning cylinders and standard procedures, and cultured in 24-well plates. Clones  
30 then are screened for morphogen expression by Western

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immunoblots using standard procedures, and morphogen expression levels compared to parental lines. Cell line stability of high expression subclones then is determined by monitoring morphogen expression levels over multiple cell passages (e.g., four or five passages).

III. Isolation of Soluble morphogen complex from conditioned media or body fluid

10

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebro-spinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

25 Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which are anticipated to be isolatable using only minor

30

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modifications of the protocol described below. An alternative protocol also envisioned to have utility an immunoaffinity column, created using standard procedures and, for example, using antibody specific  
5 for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic  
10 Press, San Diego, 1990, particularly sections VII and XI.)

In this experiment OP-1 was expressed in CHO cells as described above. The CHO cell conditioned media  
15 containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC). The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is  
20 required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1 from the bulk of the contaminating serum proteins that elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next  
25 applied to an S-Sepharose cation-exchange column equilibrated in 20 mM  $\text{NaPO}_4$  (pH 7.0) with 50 mM NaCl. This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was

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applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also may be isolated from one or more body fluids, including serum, cerebro-spinal fluid or  
5 peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M  $\text{ZnSO}_4$ . The conditioned media was  
10 titrated to pH 7.0 and applied directly to the ZN-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After  
loading the column was washed with equilibration buffer  
15 and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex is then eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

20 The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM  $\text{NaPO}_4$  (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM  $\text{NaPO}_4$  (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with  
25 an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM  $\text{NaPO}_4$  (pH 7.0). The 300 mM NaCl pool was further  
30 purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5

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mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). (see Fig. 3) The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

Figure 3 shows the absorbance profile at 280 nm. The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and

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the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP-1  
5 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 1) and a truncated form, (beginning at residue 48 of Seq. ID No. 1.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-  
10 termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 1, all of which are active as demonstrated by the standard bone induction assay.

15 V. In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be formulated from purified pro domains and mature  
20 dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions  
25 mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is  
30 decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro

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domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or  
5 dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea or GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations  
10 are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San  
15 Diego, 1990, particularly section V. Complex formation also may be aided by addition of one or more chaperone proteins.

#### VI. Stability of Soluble Morphogen Complexes

20 The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of  
25 means. Currently preferred is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 1 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal  
30 portion of other morphogens and are believed to have particular utility in enhancing complex stability for

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- all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine);
- 5 nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid; 0.01-1.0%, preferably 0.05-0.2%, including 0.1%
- 10 (v/v) nonionic detergent; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

#### VII. Activity of Soluble Morphogen Complex

- 15 Association of the pro domain with the mature dimeric species does not interfere with the morphogenic activity of the protein in vivo as demonstrated by different activity assays. Specifically, soluble OP-1 complex provided in a standard rat osteopenia model
- 20 induces significant increase in bone growth and osteocalcin production (see Table II, below), in a manner analogous to the results obtained using mature morphogen.

- 25 The assay is analogous to the osteoporosis model described in international application US92/07432 (WO93/05751), but uses aged female rats rather than ovariectomized animals. Briefly, young or aged female rats (Charles River Labs, 115-145, and 335-460g body
- 30 weight, respectively) were dosed daily for 7 days by intravenous tail injection, with either 20  $\mu\text{g/Kg}$  body weight soluble OP-1, or 100  $\mu\text{g/Kg}$  body weight soluble OP-1. Control groups of young and aged female rats were dosed only with tris-buffered saline (TBS). Water

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and food were provided to all animals ad libitum. After 14 days, animals were sacrificed, and new bone growth measured by standard histometric procedures. Osteocalcin concentrations in serum also were measured.

- 5 No detrimental effects of morphogen administration were detected as determined by changes in animal body or organ weight or by hematology profiles.

TABLE II

10	No. Animals	Animal Group	Bone Area (B.Ar/T.Ar)	Osteocalcin (ng/ml)
15	4	Control	5.50 $\pm$ 0.64	11.89 $\pm$ 4.20
20	5	Aged female, 20 $\mu$ g/Kg sol. OP-1	7.68 $\pm$ 0.63**	22.24 $\pm$ 2.28**
25	5	Aged female, 100 $\mu$ g/Kg sol. OP-1	9.82 $\pm$ 3.31*	20.87 $\pm$ 6.14*

\*P < 0.05

\*\*P < 0.01

- 30 Similar experiments performed using soluble OP-1 complex in the osteoporosis model described in WO93/05751 using ovariectomized rats also show no detrimental effect using the complex form.

- 35 Both mature and soluble morphogen also can induce CAM (cell adhesion molecule) expression, as demonstrated below. Briefly, induction of N-CAM isoforms (N-CAM-180, N-CAM-140 and N-CAM-120) can be monitored by reaction with the commercially available
- 40 antibody mAb H28.123 (Sigma Co., St. Louis) and

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available antibody mAb H28.123 (Sigma Co., St. Louis) and standard Western blot analysis (see, for example, Molecular Cloning, A Laboratory Manual, Sambrook et al. eds. Cold Spring Harbor Press, New York, 1989, particularly Section 18). Incubation of a growing culture of transformed cells of neuronal origin, NG108-15 cells (ATCC, Rockville, MD) with either mature morphogen dimers or soluble morphogen complexes (10-100 ng/ml, preferably at least 40 ng/ml) induces a redifferentiation of these cells back to a morphology characteristic of untransformed neurons, including specific induction and/or enhanced expression of all 3 N-CAM isoforms. In the experiment, cells were subcultured on poly-L-lysine coated 6-well plates and grown in chemically defined medium for 2 days before the experiment. Fresh aliquots of morphogen were added (2.5  $\mu$ l) daily.

#### VIII. Antibody Production

Provided below are standard protocols for polyclonal and monoclonal antibody production. For antibodies which recognize the soluble complex only, preferably the isolated pro region is used as the antigen; where antibodies specific to the mature protein are desired, the antigen preferably comprises at least the C-terminal domain or the intact mature sequence.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100  $\mu$ g/500  $\mu$ l of antigen, in 0.1% SDS mixed with 500  $\mu$ l Complete Freund's Adjuvant. The antigen is injected

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subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days  
5 later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against the morphogen antigen is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100  $\mu$ g of antigen and bled (15 ml per bleed) at days  
10 seven and ten after boosting.

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of the morphogen antigen. The protein or  
15 protein fragment preferably is recombinantly produced. The first injection contains 100 $\mu$ g of antigen in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50  $\mu$ g of antigen in incomplete adjuvant and is given intraperitoneally.  
20 The mouse then receives a total of 230  $\mu$ g of OP-3 in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, the mouse is boosted intraperitoneally with antigen (e.g., 100  $\mu$ g) and may be additionally boosted with a peptide  
25 fragment conjugated to bovine serum albumin with a suitable crosslinking agent. This boost can be repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells then are fused to commercially available  
30 myeloma cells at a ratio of 1:1 using PEG 1500

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(Boeringer Mannheim, Germany), and the fused cells plated and screened for mature or soluble morphogen-specific antibodies using the appropriate portion of the morphogen sequence as antigen. The cell fusion and  
5 monoclonal screening steps readily are performed according to standard procedures well described in standard texts widely available in the art.

Using these standard procedures, anti-pro domain  
10 antisera was prepared from rabbits using the isolated pro domain from OP-1 as the antigen, and monoclonal antibody ("mAb") to the mature domain was produced in mice, using an E. coli-produced truncated form of OP-1 as antigen.

15 Standard Western blot analysis performed under reducing conditions demonstrates that the anti-pro domain antisera ("anti-pro") is specific for the pro domain only, while the mAb to mature OP-1 ("anti-mature  
20 OP-1") is specific for the dimer subunits, that the two antibodies do not cross-react, and that the antibodies and can be used to distinguish between soluble and mature protein forms in a sample, e.g., of conditioned media or serum. A tabular representation of the  
25 Western blot results is in Table III below, where reactivity of mAb to mature OP-1 is indicated by "yy", and reactivity of the anti-pro antisera is indicated by "xx".

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TABLE III

	Antibody	Purified Sol OP1	Conditioned CHO Cell Media	Isolated Pro Domain	Purified Dimer Subunits
5	"anti-pro"	xx	xx	xx	
10	"anti-mature OP-1"	yy	yy		yy

15 IX. Immunoassays

The ability to detect morphogens in solution and to distinguish between soluble and mature dimeric morphogen forms provides a valuable tool for diagnostic assays, allowing one to monitor the level and type of morphogen free in the body, e.g., in serum and other body fluids, as well as to develop diagnostic and other tissue evaluating kits.

For example, OP-1 is an intimate participant in normal bone growth and resorption. Thus, soluble OP-1 is expected to be detected at higher concentrations in individuals experiencing high bone turnover, such as children, and at substantially lower levels in individuals with abnormally low rates of bone turnover, such as patients with osteoporosis, osteosarcoma, Paget's disease and the like. Monitoring the level of OP-1, or other bone targeted morphogens such as BMP2 and BMP4, in serum thus provides a means for evaluating the status of bone tissue in an individual, as well as a means for monitoring the efficacy of a treatment to regenerate damaged or lost bone tissue. Similarly,

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monitoring the level of endogenous GDF-1, can provide diagnostic information on the health of nerve tissue, particularly brain tissue. Moreover, following this disclosure one can distinguish between the level of  
5 soluble and mature forms in solution.

A currently preferred detection means for evaluating the level of morphogen in a body fluid comprises an immunoassay utilizing an antibody or other  
10 suitable binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific  
15 for that morphogen. Antibodies which recognize a morphogen protein form of interest may be generated as described herein and these antibodies then used to monitor endogenous levels of protein in a body fluid, such as serum, whole blood or peritoneal fluid. To  
20 monitor endogenous concentrations of soluble morphogen, the antibody chosen preferably has binding specificity for the soluble form e.g., has specificity for the pro domain. Such antibodies may be generated by using the pro domain or a portion thereof as the antigen,  
25 essentially as described herein. A suitable pro domain for use as an antigen may be obtained by isolating the soluble complex and then separating the noncovalently associated pro domain from the mature domain using standard procedures, e.g., by passing the complex over  
30 an HPLC column, as described above or by separation by gel electrophoresis. Alternatively, the pro form of the protein in its monomeric form may be used as the

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antigen and the candidate antibodies screened by Western blot or other standard immunoassay for those which recognize the pro domain of the soluble form of the protein of interest, but not the mature form, also  
5 as described above.

Monomeric pro forms can be obtained from cell lysates of CHO produced cells, or from prokaryotic expression of a DNA encoding the pro form, in for  
10 example, E.coli. The pro form, which has an apparent molecular weight of about 50 kDa in mammalian cells, can then be isolated by HPLC and/or by gel electrophoresis, as described above.

15 In order to detect and/or quantitate the amount of morphogenic protein present in a solution, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. Here, soluble and mature forms of the  
20 morphogen also may be distinguished by using antibodies that discriminate between the two forms of the proteins as described above. Currently preferred assays include ELISAS and radioimmunassays, including standard competitor assays useful for quantitating the morphogen  
25 in a sample, where an unknown amount of sample morphogen is allowed to react with anti-morphogen antibody and this interaction is competed with a known amount of labeled antigen. The level of bound or free labeled antigen at equilibrium then is measured to  
30 quantitate the amount of unlabeled antigen in solution, the amount of sample antigen being proportional to the amount of free labeled antigen. Exemplary protocols for these assays are provided below. However, as will be appreciated by those skilled in the art, variations

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- of these protocols, as well as other immunoassays, are well known in the literature and within the skill of the art. For example, in the ELISA protocol provided below, soluble OP-1 is identified in a sample using
- 5 biotinylated anti-pro antiserum. Biotinylated antibodies can be visualized in a colormetric assay or in a chemiluminescent assay, as described below. Alternatively, the antibody can be radio-labeled with a suitable molecule, such as  $^{125}\text{I}$ . Still another
- 10 protocol that may be used is a solid phase immunoassay, preferably using an affinity column with anti-morphogen antibody complexed to the matrix surface and over which a serum sample may be passed. A detailed description of useful immunoassays, including protocols and general
- 15 considerations is provided in, for example, Molecular Cloning: A Laboratory Manual, Sambrook et al., eds. Cold Spring Harbor Press, New York, 1989, particularly Section 18.
- 20 For serum assays, the serum preferably first is partially purified to remove some of the excess, contaminating serum proteins, such as serum albumin. Preferably the serum is extracted by precipitation in ammonium sulfate (e.g., 45%) such that the complex is
- 25 precipitated. Further purification can be achieved using purification strategies that take advantage of the differential solubility of soluble morphogen complex or mature morphogens relative to that of the other proteins present in serum. Further purification
- 30 also can be achieved by chromatographic techniques well known in the art.

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Soluble OP-1 may be detected using a polyclonal antibody specific for the OP-1 pro domain in an ELISA, as follows. 1  $\mu\text{g}/100\ \mu\text{l}$  of affinity-purified polyclonal rabbit IgG specific for OP-1-pro is added to  
5 each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely  
10 with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100  $\mu\text{l}$  aliquot of an appropriate dilution of each of the test samples of cell culture supernatant or serum  
15 sample is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100  $\mu\text{l}$  biotinylated rabbit anti-pro serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at  
20 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100  $\mu\text{l}$  strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to  
25 each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50  $\mu\text{l}$  substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15  
30 min. Then, 50  $\mu\text{l}$  amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50  $\mu\text{l}$  0.3 M sulphuric acid.

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The OD at 490 nm of the solution in each well is recorded. To quantitate the level of soluble OP-1 in the sample, a standard curve is performed in parallel with the test samples. In the standard curve, known  
5 increasing amounts of purified OP-1-pro is added. Alternatively, using, for example, Lumi-phos 530 (Analytical Luminescence Laboratories) as the substrate and detection at 300-650 nm in a standard luminometer, complexes can be detected by chemiluminescence, which  
10 typically provides a more sensitive assay than detection by means of a visible color change.

Morphogen (soluble or mature form) may be detected in a standard plated-based radioimmunoassay as follows.  
15 Empirically determined limiting levels of anti-morphogen antibody (e.g., anti-OP-1, typically 50-80 ng/well) are bound to wells of a PVC plate e.g., in 50  $\mu$ l PBS phosphate buffered saline. After sufficient incubation to allow binding at room  
20 temperature, typically one hour, the plate is washed in a PBS/Tween 20 solution, ("washing buffer"), and 200  $\mu$ l of block (3% BSA, 0.1 $\mu$  lysine in 1xBSB) is added to each well and allowed to incubate for 1 hour, after which the wells are washed again in washing buffer. 40  
25  $\mu$ l of a sample composed of serially diluted plasma (preferably partially purified as described above) or morphogen standard (e.g., OP-1) is added to wells in triplicate. Samples preferably are diluted in PTH (15 mM  $\text{KH}_2\text{PO}_4$ , 8 mM  $\text{Na}_2\text{PO}_4$ , 27 mM KCl, 137 mM NaCl,  
30 0.05% Tween 20, 1 mg/ml HSA, 0.05%  $\text{NaN}_3$ , pH 7.2). 10  $\mu$ l of labelled competitor antigen, preferably 100,000-500,000 cpm/sample is added (e.g.,  $^{125}\text{I}$  OP-1, radiolabelled using standard procedures), and plates are incubated overnight at 4°C. Plates then are washed

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in washing buffer, and allowed to dry. Wells are cut apart and bound labelled OP-1 counted in a standard gamma counter. The quantities of bound labelled antigen (e.g.,  $^{125}\text{I}$  OP-1) measured in the presence and  
5 absence of sample then are compared, the difference being proportional to the amount of sample antigen (morphogen) present in the sample fluid.

As a corollary assay method, immunoassays may be  
10 developed to detect endogenous anti-morphogen antibodies, and to distinguish between such antibodies to soluble or mature forms. Endogenous anti-morphogen antibodies have been detected in serum, and their level is known to increase, for example, upon implanting of  
15 an osteogenic device in a mammal. Without being limited to a particular theory, these antibodies may play a role in modulating morphogen activity by modulating the level of available protein in serum. Assays that monitor the level of endogenous antibodies  
20 in blood or their body fluids thus can be used in diagnostic assays to evaluate the status of a tissue, as well as to provide a means for monitoring the efficacy of a therapy for tissue regeneration.

25 The currently preferred means for detecting endogenous anti-morphogen antibodies is by means of a standard Western blot. See, for example, Molecular Cloning: A Laboratory Manual Sambrook et al., eds., Cold Spring Harbor Press, New York, 1989, particularly  
30 pages 18.60-18.75, incorporated herein by reference, for a detailed description of these assays. Purified mature or soluble morphogen is electrophoresed on an SDS polyacrylamide gel under oxidized or reduced conditions designed to separate the proteins in

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solution, and the proteins then transferred to a polyvinylidene difluoride microporus membrane (0.45  $\mu$ m pore sizes) using standard buffers and procedures. The filter then is incubated with the  
5 serum being tested (at various dilutions). Antibodies bound to either the pro domain or the mature morphogen domain are detected by means of an anti-human antibody protein, e.g., goat anti-human Ig. Titers of the antimorphogen antibodies can be determined by further  
10 dilution of the serum until no signal is detected.

X. Formulations and Methods for Administering Soluble Morphogens as Therapeutic Agents

15 The soluble morphogens of this invention are particularly useful as therapeutic agents to regenerate diseased or damaged tissue in a mammal, particularly a human.

20 The soluble morphogen complexes may be used to particular advantage in regeneration of damaged or diseased lung, heart, liver, kidney, nerve or pancreas tissue, as well as in the transplantation and/or grafting of these tissues and bone marrow, skin,  
25 gastrointestinal mucosa, and other living tissues.

The soluble morphogen complexes described herein may be provided to an individual by any suitable means, preferably directly or systemically, e.g., parenterally  
30 or orally. Where the morphogen is to be provided directly (e.g., locally, as by injection, to a desired tissue site), or parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular,

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intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the soluble morphogen complex preferably comprises part of an aqueous solution. The  
5 solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the soluble morphogen thus may  
10 comprise normal physiologic saline (0.9% NaCl, 0.15M), pH 7-7.4.

Soluble morphogens of this invention are readily purified from cultured cell media into a physiological  
15 buffer, as described above. In addition, and as described above, if desired, the soluble complexes may be formulated with one or more additional additives, including basic amino acids (e.g., L-arginine, lysine, betaine); non-ionic detergents (e.g. Tween-80 or  
20 NonIdet-120) and carrier proteins (e.g., serum albumin and casein).

Useful solutions for oral or parenteral administration may be prepared by any of the methods  
25 well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences, (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin,  
30 hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity.

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Biocompatible, preferably bioresorbable polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, polylactide, polyglycolide and lactide/glycolide copolymers, may be  
5 useful excipients to control the release of the soluble morphogen in vivo.

Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl  
10 acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl  
15 ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally.

The soluble morphogens described herein also may be  
20 administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins readily are degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the mature  
25 domains of the morphogens described herein typically are acid-stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified, in mammary gland extract, colostrum and milk, as well as  
30 saliva. Moreover, the OP-1 purified from mammary gland extract is morphogenically active. For example, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone

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assay, such as is disclosed in U.S. Pat. No. 4,968,590. In addition, endogenous morphogen also is detected in human serum (see above). Finally, comparative experiments with soluble and mature morphogens in a number of experiments defining morphogenic activity indicate that the non-covalent association of the pro domain with the dimeric species does not interfere with morphogenic activity. These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual, and that soluble morphogens have utility in systemic administration protocols.

The soluble complexes provided herein also may be associated with molecules capable of targeting the morphogen to a desired tissue. For example, tetracycline and diphosphonates (bisphosphonates) are known to bind to bone mineral, particularly at zones of bone remodeling, when they are provided systemically in a mammal. Accordingly, these molecules may be included as useful agents for targeting soluble morphogens to bone tissue. Alternatively, an antibody or other binding protein that interacts specifically with a surface molecule on the desired target tissue cells also may be used. Such targeting molecules further may be covalently associated to the morphogen complex, e.g., by chemical crosslinking, or by using standard genetic engineering means to create, for example, an acid labile bond such as an Asp-Pro linkage. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

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Finally, the soluble morphogen complexes provided herein may be administered alone or in combination with other molecules known to have a beneficial effect on tissue morphogenesis, including molecules capable of

5 tissue repair and regeneration and/or inhibiting inflammation. Examples of useful cofactors for stimulating bone tissue growth in osteoporotic individuals, for example, include but are not limited to, vitamin D<sub>3</sub>, calcitonin, prostaglandins, parathyroid

10 hormone, dexamethasone, estrogen and IGF-I or IGF-II. Useful cofactors for nerve tissue repair and regeneration may include nerve growth factors. Other useful cofactors include symptom-alleviating cofactors, including antiseptics, antibiotics, antiviral and

15 antifungal agents and analgesics and anesthetics.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and

20 carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of

25 powders, nasal drops or aerosols. Where adhesion to a tissue surface is desired the composition may include the morphogen dispersed in a fibrinogen-thrombin composition or other bioadhesive such as is disclosed, for example in PCT US91/09275, the disclosure of which

30 is incorporated herein by reference. The composition then may be painted, sprayed or otherwise applied to the desired tissue surface.

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The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations of the morphogen to target tissue for a time sufficient to induce morphogenesis, including particular steps thereof, as described above.

Where the soluble morphogen complex is to be used as part of a transplant procedure, the morphogen may be provided to the living tissue or organ to be transplanted prior to removal of the tissue or organ from the donor. The morphogen may be provided to the donor host directly, as by injection of a formulation comprising the soluble complex into the tissue, or indirectly, e.g., by oral or parenteral administration, using any of the means described above.

Alternatively or, in addition, once removed from the donor, the organ or living tissue may be placed in a preservation solution containing the morphogen. In addition, the recipient also preferably is provided with the morphogen just prior to, or concomitant with, transplantation. In all cases, the soluble complex may be administered directly to the tissue at risk, as by injection to the tissue, or it may be provided systemically, either by oral or parenteral administration, using any of the methods and formulations described herein and/or known in the art.

Where the morphogen comprises part of a tissue or organ preservation solution, any commercially available preservation solution may be used to advantage. A

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useful preservation solution is described in in PCT/US92/07358 (W093/04692), incorporated herein by reference.

- 5       As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g.,
- 10   hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of tissue loss or defect, the overall health status of the particular
- 15   patient, the relative biological efficacy of the compound selected, the formulation of the compound, the presence and types of excipients in the formulation, and the route of administration. In general terms, the compounds of this invention may be provided in an
- 20   aqueous physiological buffer solution containing about 0.001 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1  $\mu$ g/kg to
- 25   100 mg/kg of body weight. No obvious morphogen-induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20  $\mu$ g) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10  $\mu$ g systemic injections of morphogen (e.g., OP-1) injected
- 30   daily for 10 days into normal newborn mice does not produce any gross abnormalities.

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Where morphogens are administered systemically, in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

#### Other Embodiments

10

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

15

20

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## SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT:
- (A) NAME: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 35 SOUTH STREET
- 10 (C) CITY: HOPKINTON
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- (G) TELEPHONE: 1-508-435-9001
- 15 (H) TELEFAX: 1-508-435-0454
- (I) TELEX:
- (ii) TITLE OF INVENTION: NOVEL MORPHOGENIC PROTEIN COMPOSITIONS  
OF MATTER
- 20 (iii) NUMBER OF SEQUENCES: 23
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: PATENT ADMINISTRATOR/CREATIVE BIOMOLECULES,  
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- (D) STATE: MA
- (E) COUNTRY: USA
- 30 (F) ZIP: 01748
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- 40 (B) FILING DATE:
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER:
- 45 (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: KELLEY, ROBIN, D.
- (B) REGISTRATION NUMBER: 34,637
- 50 (C) REFERENCE/DOCKET NUMBER: CRP-081CP

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## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1822 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: HOMO SAPIENS  
 (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 49..1341  
 (C) IDENTIFICATION METHOD: experimental  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "OP1"  
 /evidence= EXPERIMENTAL  
 /standard\_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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		1
35	CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA	105
	Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	
	5 10 15	
40	CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
	Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asn	
	20 25 30 35	
45	GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
	Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
	40 45 50	
	CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
	Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
	55 60 65	
50		

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	CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	TTC	ATG	297
	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	
			70					75					80				
5	CTG	GAC	CTG	TAC	AAC	GCC	ATG	CGC	GTG	GAG	GAG	GGC	GGC	GGG	CCC	GGC	345
	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	Pro	Gly	
		85					90					95					
10	GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	393
	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	
	100					105					110					115	
15	CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
					120					125					130		
20	ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
				135					140					145			
25	CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
			150				155						160				
30	CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
			165				170					175					
35	TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
	180					185					190					195	
40	CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200					205						210		
45	GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
				215					220					225			
50	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
			230				235						240				
55	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
			245				250					255					

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	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC	873
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro	
	260 265 270 275	
5	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC	921
	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile	
	280 285 290	
10	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC	969
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro	
	295 300 305	
15	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC	1017
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser	
	310 315 320	
20	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065
	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe	
	325 330 335	
25	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
	340 345 350 355	
30	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
	360 365 370	
35	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
	375 380 385	
40	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257
	Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala	
	390 395 400	
45	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305
	Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys	
	405 410 415	
50	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC	1351
	Tyr Arg Asn Met Val Arg Ala Cys Gly Cys His	
	420 425 430	
55	GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
	GAACCAAGCAG ACCAACTGCC TTTTGTGAGA CTTTCCCCTC CCTATCCCCA ACTTTAAAGG	1471
	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531

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ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591  
 GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651  
 5 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711  
 GCGGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAAATTGAC CCGGAAGTTC 1771  
 CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A 1822  
 10

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 15 (A) LENGTH: 431 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15  
 25 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20 25 30  
 30 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45  
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60  
 35 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
 65 70 75 80  
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly  
 85 90 95  
 40 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser  
 100 105 110  
 45 Thr Gln Gly Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr  
 115 120 125  
 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys  
 130 135 140

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Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu  
 145 150 155 160  
 5 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile  
 165 170 175  
 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile  
 180 185 190  
 10 Ser Val Tyr Gln Val Leu Gln His Leu Gly Arg Glu Ser Asp Leu  
 195 200 205  
 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu  
 210 215 220  
 15 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg  
 225 230 235 240  
 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser  
 245 250 255  
 20 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn  
 260 265 270  
 25 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe  
 275 280 285  
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser  
 290 295 300  
 30 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu  
 305 310 315 320  
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr  
 325 330 335  
 35 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu  
 340 345 350  
 40 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn  
 355 360 365  
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His  
 370 375 380  
 45 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln  
 385 390 395 400

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Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile  
 405 410 415

5 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 1873 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 15 (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- 20 (A) NAME/KEY: CDS  
 (B) LOCATION: 104..1393  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "MOP1"  
 /note= "MOP1 CDNA"

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

30 CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115  
 Met His Val Arg  
 1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163  
 35 Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro  
 5 10 15 20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211  
 40 Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu  
 25 30 35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG 259  
 Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg  
 40 45 50

45 GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG 307  
 Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro  
 55 60 65

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	CGC	CCG	CAC	CTC	CAG	GGA	AAG	CAT	AAT	TCG	GCG	CCC	ATG	TTC	ATG	TTG	355
	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	Leu	
		70					75					80					
5	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	AGC	GGG	CCG	GAC	GGA	CAG	403
	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Ser	Gly	Pro	Asp	Gly	Gln	
	85					90					95				100		
10	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
					105					110					115		
15	TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
				120					125					130			
20	ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
				135				140					145				
25	CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
	150					155						160					
30	GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
	Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
	165					170				175						180	
35	CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185						190					195		
40	CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
				200					205					210			
45	CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
			215					220					225				
50	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
			230				235					240					
55	CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
	245					250					255					260	



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GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT 1653  
 GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTGAGGAGT 1713  
 5 AATCGCAAGC CTCGTTACGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCCTGGCG 1773  
 TCTGTGTTGA AGGGAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAACCCAT 1833  
 GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC 1873

10

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 15 (A) LENGTH: 430 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein  
 20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15  
 25 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20 25 30  
 30 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45  
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60  
 35 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
 65 70 75 80  
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly  
 85 90 95  
 40 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr  
 100 105 110  
 45 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp  
 115 120 125  
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu  
 130 135 140

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Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser  
 145 150 155 160  
 5 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr  
 165 170 175  
 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr  
 180 185 190  
 10 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe  
 195 200 205  
 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val  
 210 215 220  
 15 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His  
 225 230 235 240  
 20 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile  
 245 250 255  
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys  
 260 265 270  
 25 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg  
 275 280 285  
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys  
 290 295 300  
 30 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn  
 305 310 315 320  
 35 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val  
 325 330 335  
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly  
 340 345 350  
 40 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser  
 355 360 365  
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe  
 370 375 380  
 45 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu  
 385 390 395 400

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Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu  
 405 410 415

5 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

## (2) INFORMATION FOR SEQ ID NO:5:

10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1723 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens  
 (F) TISSUE TYPE: HIPPOCAMPUS

20 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 490..1696  
 25 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "hOP2-PP"  
 /note= "hOP2 (cDNA)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGTTG GAGCAGGAGG TGGCACGGCA 60  
 GGGCTGGAGG GCTCCCTATG ACTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC 120  
 35 CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCCATC GCCCCTGCGC TGCTCGGACC 180  
 GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT 240  
 CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG 300  
 40 GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCCTCC 360  
 CGCCCCGCCC CGCCGCCCCG CGCCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC 420  
 45 AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC 480  
 CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG 528  
 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu  
 1 5 10

50

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	GCG	CTA	TGC	GCG	CTG	GGC	GGG	GGC	GGC	CCC	GGC	CTG	CGA	CCC	CCG	CCC	576
	Ala	Leu	Cys	Ala	Leu	Gly	Gly	Gly	Gly	Pro	Gly	Leu	Arg	Pro	Pro	Pro	
	15					20					25						
5	GGC	TGT	CCC	CAG	CGA	CGT	CTG	GGC	GCG	CGC	GAG	CGC	CGG	GAC	GTG	CAG	624
	Gly	Cys	Pro	Gln	Arg	Arg	Leu	Gly	Ala	Arg	Glu	Arg	Arg	Asp	Val	Gln	
	30					35					40				45		
10	CGC	GAG	ATC	CTG	GCG	GTG	CTC	GGG	CTG	CCT	GGG	CGG	CCC	CGG	CCC	CGC	672
	Arg	Glu	Ile	Leu	Ala	Val	Leu	Gly	Leu	Pro	Gly	Arg	Pro	Arg	Pro	Arg	
					50					55					60		
15	GCG	CCA	CCC	GCC	GCC	TCC	CGG	CTG	CCC	GCG	TCC	GCG	CCG	CTC	TTC	ATG	720
	Ala	Pro	Pro		65	Ala	Ser	Arg	Leu	Pro	Ala	Ser	Ala	Pro	Leu	Phe	
									70					75		Met	
20	CTG	GAC	CTG	TAC	CAC	GCC	ATG	GCC	GGC	GAC	GAC	GAC	GAG	GAC	GGC	GCG	768
	Leu	Asp	Leu	Tyr	His	Ala	Met	Ala	Gly	Asp	Asp	Asp	Glu	Asp	Gly	Ala	
				80				85					90				
	CCC	GCG	GAG	CGG	CGC	CTG	GGC	CGC	GCC	GAC	CTG	GTC	ATG	AGC	TTC	GTT	816
	Pro	Ala	Glu	Arg	Arg	Leu	Gly	Arg	Ala	Asp	Leu	Val	Met	Ser	Phe	Val	
	95					100						105					
25	AAC	ATG	GTG	GAG	CGA	GAC	CGT	GCC	CTG	GGC	CAC	CAG	GAG	CCC	CAT	TGG	864
	Asn	Met	Val	Glu	Arg	Asp	Arg	Ala	Leu	Gly	His	Gln	Glu	Pro	His	Trp	
	110					115					120					125	
30	AAG	GAG	TTC	CGC	TTT	GAC	CTG	ACC	CAG	ATC	CCG	GCT	GGG	GAG	GCG	GTC	912
	Lys	Glu	Phe	Arg	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	
					130					135					140		
35	ACA	GCT	GCG	GAG	TTC	CGG	ATT	TAC	AAG	GTG	CCC	AGC	ATC	CAC	CTG	CTC	960
	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Val	Pro	Ser	Ile	His	Leu	Leu	
				145				150					155				
40	AAC	AGG	ACC	CTC	CAC	GTC	AGC	ATG	TTC	CAG	GTG	GTC	CAG	GAG	CAG	TCC	1008
	Asn	Arg	Thr	Leu	His	Val	Ser	Met	Phe	Gln	Val	Val	Gln	Glu	Gln	Ser	
				160				165					170				
	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	CTC	CGA	GCT	1056
	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ala	
	175					180						185					
45	GGA	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAT	GTC	ACA	GCA	GCC	AGT	GAC	TGC	1104
	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Val	Thr	Ala	Ala	Ser	Asp	Cys	
	190					195					200					205	



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## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 402 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
1 5 10 15

15 Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro  
20 25 30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile  
35 40 45

20 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro  
50 55 60

25 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu  
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu  
85 90 95

30 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val  
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe  
115 120 125

35 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala  
130 135 140

40 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr  
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu  
165 170 175

45 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu  
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu  
195 200 205

50

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Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp  
 210 215 220  
 5 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala  
 225 230 235 240  
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro  
 245 250 255  
 10 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln  
 260 265 270  
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile  
 275 280 285  
 15 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His  
 290 295 300  
 20 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile  
 305 310 315 320  
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe  
 325 330 335  
 25 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser  
 340 345 350  
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala  
 355 360 365  
 30 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn  
 370 375 380  
 35 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly  
 385 390 395 400  
 Cys His

## 40 (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE  
 (F) TISSUE TYPE: EMBRYO

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## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 93..1289

(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"

5 /product= "mOP2-PP"

/note= "mOP2 cDNA"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10 GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT 60

ACCACTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA 113  
Met Ala Met Arg Pro Gly Pro

15 1 5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT 161  
Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly

20 10 15 20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG 209  
Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu

25 25 30 35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA 257  
Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly

40 45 50 55

CGC CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC 305  
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser

30 60 65 70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC 353  
Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp

35 75 80 85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG 401  
Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met

40 90 95 100

AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG 449  
Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu

105 110 115

45 CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG 497  
Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly

120 125 130 135

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	GAG	GCT	GTC	ACA	GCT	GCT	GAG	TTC	CGG	ATC	TAC	AAA	GAA	CCC	AGC	ACC	545
	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	
				140					145						150		
5	CAC	CCG	CTC	AAC	ACA	ACC	CTC	CAC	ATC	AGC	ATG	TTC	GAA	GTG	GTC	CAA	593
	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	Ser	Met	Phe	Glu	Val	Val	Gln	
				155					160					165			
10	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	641
	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	
			170					175					180				
15	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	ACA	GCA	GCC	689
	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	Thr	Ala	Ala	
		185					190					195					
20	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	737
	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	
		200				205					210				215		
	TAT	GTG	GAA	ACC	GGC	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	CTG	GCT	GGT	785
	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	Leu	Ala	Gly	
				220						225				230			
25	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	ATG	GTA	ACC	833
	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	Met	Val	Thr	
			235					240						245			
30	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	GCA	GCG	AGA	881
	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	Ala	Ala	Arg	
			250					255					260				
35	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	CCG	CAC	CCC	929
	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	Pro	
		265					270					275					
40	AAC	AAA	CTC	CCA	GGG	ATC	TTT	GAT	GAT	GGC	CAC	GGT	TCC	CGC	GGC	AGA	977
	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	
		280				285				290					295		
	GAG	GTT	TGC	CGC	AGG	CAT	GAG	CTC	TAC	GTC	AGC	TTC	CGT	GAC	CTT	GGC	1025
	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	
				300					305						310		
45	TGG	CTG	GAC	TGG	GTC	ATC	GCC	CCC	CAG	GGC	TAC	TCT	GCC	TAT	TAC	TGT	1073
	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	
				315					320					325			

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	GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
	Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
	330 335 340	
5	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
10	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
15	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC TGCTTCTACT	1319
	Val Val Lys Ala Cys Gly Cys His	
	395	
20	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA AAATTCTGGT	1439
25	CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGCCATC CTCAGCCAC	1619
30	AATGGCAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGT	1679
	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTTAGGT ATAACAGACA CATACTTA	1739
35	GATCAATGCA TCGTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
	CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
	CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAC	1919
40	GGAATTC	1926

## (2) INFORMATION FOR SEQ ID NO:8:

45

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

50

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

5 Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
    1                  5                  10                  15  
   Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln  
                   20                  25                  30  
 10 Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu  
                   35                  40                  45  
   Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala  
    15                  50                  55                  60  
   Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr  
    65                  70                  75                  80  
 20 His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu  
                   85                  90                  95  
   Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp  
                   100                  105                  110  
 25 Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp  
                   115                  120                  125  
   Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg  
    30                  130                  135                  140  
   Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile  
    145                  150                  155                  160  
 35 Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu  
                   165                  170                  175  
   Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu  
                   180                  185                  190  
 40 Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His  
                   195                  200                  205  
   Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser  
    45                  210                  215                  220  
   Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser  
    225                  230                  235                  240

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	Arg	Gln	Pro	Phe	Met	Val	Thr	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val
					245					250					255	
5	Arg	Ala	Pro	Arg	Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys
				260					265					270		
	Thr	Asn	Glu	Leu	Pro	His	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp
				275				280					285			
10	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr
		290					295					300				
	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln
	305					310					315					320
15	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asp
				325						330					335	
	Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His
				340					345					350		
	Leu	Met	Lys	Pro	Asp	Val	Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
			355					360					365			
25	Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile
		370					375					380				
	Leu	Arg	Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala	Cys	Gly	Cys	His	
	385					390					395					

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

**(ix) FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..399  
(D) OTHER INFORMATION: /note= "PRE-PRO-OP3 (MOUSE)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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	Met	Ala	Ala	Arg	Pro	Gly	Leu	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
	1				5					10					15	
5	Val	Leu	Gly	Gly	Gly	His	Leu	Ser	His	Pro	Pro	His	Val	Phe	Pro	Gln
			20					25					30			
	Arg	Arg	Leu	Gly	Val	Arg	Glu	Pro	Arg	Asp	Met	Gln	Arg	Glu	Ile	Arg
			35					40					45			
10	Glu	Val	Leu	Gly	Leu	Ala	Gly	Arg	Pro	Arg	Ser	Arg	Ala	Pro	Val	Gly
		50					55					60				
	Ala	Ala	Gln	Gln	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr
	65					70					75					80
15	Arg	Ala	Met	Thr	Asp	Asp	Ser	Gly	Gly	Gly	Thr	Pro	Gln	Pro	His	Leu
					85					90					95	
	Asp	Arg	Ala	Asp	Leu	Ile	Met	Ser	Phe	Val	Asn	Ile	Val	Glu	Arg	Asp
20				100					105					110		
	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp
			115				120						125			
25	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg
		130					135					140				
	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile
	145					150					155					160
30	Ser	Met	Phe	Glu	Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu
				165						170					175	
	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu
35				180					185					190		
	Val	Leu	Asp	Ile	Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His
			195					200					205			
40	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	Thr	Glu	Asp	Gly	His	Ser
		210					215					220				
	Ile	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser
	225					230					235					240
45	Arg	Gln	Pro	Phe	Met	Val	Gly	Phe	Phe	Arg	Ala	Asn	Gln	Ser	Pro	Val
					245					250					255	

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Arg Ala Pro Arg Thr Ala Arg Pro Leu Lys Lys Lys Gln Leu Asn Gln  
                                 260                                265                                270  
 5 Ile Asn Gln Leu Pro His Ser Asn Lys His Leu Gly Ile Leu Asp Asp  
                                 275                                280                                285  
 Gly His Gly Ser His Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr  
                                 290                                295                                300  
 10 Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Ser Val Ile Ala Pro Gln  
                                 305                                310                                315                                320  
 Gly Tyr Ser Ala Tyr Tyr Cys Ala Gly Glu Cys Ile Tyr Pro Leu Asn  
                                 325                                330                                335  
 15 Ser Cys Met Asn Ser Thr Asn His Ala Thr Met Gln Ala Leu Val His  
                                 340                                345                                350  
 20 Leu Met Lys Pro Asp Ile Ile Pro Lys Val Cys Cys Val Pro Thr Glu  
                                 355                                360                                365  
 Leu Ser Ala Ile Ser Leu Leu Tyr Tyr Asp Arg Asn Asn Asn Val Ile  
                                 370                                375                                380  
 25 Leu Arg Arg Glu Arg Asn Met Val Val Gln Ala Cys Gly Cys His  
                                 385                                390                                395

## (2) INFORMATION FOR SEQ ID NO:10:

- 30 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 396 amino acids  
     (B) TYPE: amino acid  
     (C) STRANDEDNESS: single  
     (D) TOPOLOGY: linear  
 35  
 35 (ii) MOLECULE TYPE: protein  
  
 40 (ix) FEATURE:  
     (A) NAME/KEY: Protein  
     (B) LOCATION: 1..396  
     (D) OTHER INFORMATION: /note= "PRE-PRO-BMP2 (HUMAN)"  
  
 45 (x) PUBLICATION INFORMATION:  
     (A) AUTHORS: WOZNEY,  
     (C) JOURNAL: SCIENCE  
     (D) VOLUME: 242  
     (F) PAGES: 1528-1534  
     (G) DATE: 1988  
 50

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5	Met	Val	Ala	Gly	Thr	Arg	Cys	Leu	Leu	Ala	Leu	Leu	Leu	Pro	Gln	Val	1	5	10	15	
	Leu	Leu	Gly	Gly	Ala	Ala	Gly	Leu	Val	Pro	Glu	Leu	Gly	Arg	Arg	Lys	20	25	30		
10	Phe	Ala	Ala	Ala	Ser	Ser	Gly	Arg	Pro	Ser	Ser	Gln	Pro	Ser	Asp	Glu	35	40	45		
	Val	Leu	Ser	Glu	Phe	Glu	Leu	Arg	Leu	Leu	Ser	Met	Phe	Gly	Leu	Lys	50	55	60		
15	Gln	Arg	Pro	Thr	Pro	Ser	Arg	Asp	Ala	Val	Val	Pro	Pro	Tyr	Met	Leu	65	70	75	80	
	Asp	Leu	Tyr	Arg	Arg	His	Ser	Gly	Gln	Pro	Gly	Ser	Pro	Ala	Pro	Asp	85	90	95		
20	His	Arg	Leu	Glu	Arg	Ala	Ala	Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	100	105	110		
	His	His	Glu	Glu	Ser	Leu	Glu	Glu	Leu	Pro	Glu	Thr	Ser	Gly	Lys	Thr	115	120	125		
25	Thr	Arg	Arg	Phe	Phe	Phe	Asn	Leu	Ser	Ser	Ile	Pro	Thr	Glu	Glu	Phe	130	135	140		
30	Ile	Thr	Ser	Ala	Glu	Leu	Gln	Val	Phe	Arg	Glu	Gln	Met	Gln	Asp	Ala	145	150	155	160	
	Leu	Gly	Asn	Asn	Ser	Ser	Phe	His	His	Arg	Ile	Asn	Ile	Tyr	Glu	Ile	165	170	175		
35	Ile	Lys	Pro	Ala	Thr	Ala	Asn	Ser	Lys	Phe	Pro	Val	Thr	Arg	Leu	Leu	180	185	190		
40	Asp	Thr	Arg	Leu	Val	Asn	Gln	Asn	Ala	Ser	Arg	Trp	Glu	Ser	Phe	Asp	195	200	205		
	Val	Thr	Pro	Ala	Val	Met	Arg	Trp	Thr	Ala	Gln	Gly	His	Ala	Asn	His	210	215	220		
45	Gly	Phe	Val	Val	Glu	Val	Ala	His	Leu	Glu	Glu	Lys	Gln	Gly	Val	Ser	Lys	225	230	235	240
	Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu	His	Gln	Asp	Glu	His	Ser	Trp	245	250	255		
50																					

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Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys Gly  
 260 265 270  
 5 His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg  
 275 280 285  
 Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe  
 290 295 300 305  
 10 Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His  
 310 315 320  
 Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu  
 325 330 335  
 15 Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn  
 340 345 350  
 Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 355 360 365 370  
 20 Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr  
 375 380 385  
 25 Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg  
 390 395

## (2) INFORMATION FOR SEQ ID NO:11:

- 30 (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 408 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 35 (ii) MOLECULE TYPE: protein  
 (ix) FEATURE:  
 40 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..408  
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP4 (HUMAN)"  
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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	Met	Ile	Pro	Gly	Asn	Arg	Met	Leu	Met	Val	Val	Leu	Leu	Cys	Gln	Val
	1				5					10					15	
5	Leu	Leu	Gly	Gly	Ala	Ser	His	Ala	Ser	Leu	Ile	Pro	Glu	Thr	Gly	Lys
			20						25					30		
	Lys	Lys	Val	Ala	Glu	Ile	Gln	Gly	His	Ala	Gly	Gly	Arg	Arg	Ser	Gly
			35					40					45			
10	Gln	Ser	His	Glu	Leu	Leu	Arg	Asp	Phe	Glu	Ala	Thr	Leu	Leu	Gln	Met
		50					55					60				
	Phe	Gly	Leu	Arg	Arg	Arg	Pro	Gln	Pro	Ser	Lys	Ser	Ala	Val	Ile	Pro
	65					70					75					80
15	Asp	Tyr	Met	Arg	Asp	Leu	Tyr	Arg	Leu	Gln	Ser	Gly	Glu	Glu	Glu	Glu
				85						90					95	
	Glu	Gln	Ile	His	Ser	Thr	Gly	Leu	Glu	Tyr	Pro	Glu	Arg	Pro	Ala	Ser
20				100					105					110		
	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His	His	Glu	Glu	His	Leu	Glu	Asn
			115					120					125			
25	Ile	Pro	Gly	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arg	Phe	Leu	Phe	Asn	Leu
		130					135					140				
	Ser	Ser	Ile	Pro	Glu	Asn	Glu	Val	Ile	Ser	Ser	Ala	Glu	Leu	Arg	Leu
30		145				150					155					160
	Phe	Arg	Glu	Gln	Val	Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His
				165						170					175	
	Arg	Ile	Asn	Ile	Tyr	Glu	Val	Met	Lys	Pro	Pro	Ala	Glu	Val	Val	Pro
35				180					185					190		
	Gly	His	Leu	Ile	Thr	Arg	Leu	Leu	Asp	Thr	Arg	Leu	Val	His	His	Asn
			195				200						205			
40	Val	Thr	Arg	Trp	Glu	Thr	Phe	Asp	Val	Ser	Pro	Ala	Val	Leu	Arg	Trp
		210					215					220				
	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu	Val	Thr	His
	225					230					235					240
45	Leu	His	Gln	Thr	Arg	Thr	His	Gln	Gly	Gln	His	Val	Arg	Ile	Ser	Arg
					245					250					255	

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Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu  
 260 265 270  
 5 Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg  
 275 280 285  
 Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys  
 290 295 300  
 10 Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Phe Asp  
 305 310 315 320  
 Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe  
 325 330 335  
 15 Tyr Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser  
 340 345 350  
 Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser  
 355 360 365  
 Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met  
 370 375 380  
 25 Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 385 390 395  
 Met Val Val Glu Gly Cys Gly Cys Arg  
 400 405

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..588  
 (D) OTHER INFORMATION: /note= "PRE-PRO-DPP"

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## (x) PUBLICATION INFORMATION:

- 5 (A) AUTHORS: PADGETT,  
 (C) JOURNAL: NATURE  
 (D) VOLUME: 325  
 (F) PAGES: 81-84  
 (G) DATE: 1987

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

10 Met Arg Ala Trp Leu Leu Leu Leu Ala Val Leu Ala Thr Phe Gln Thr  
 1 5 10 15  
 Ile Val Arg Val Ala Ser Thr Glu Asp Ile Ser Gln Arg Phe Ile Ala  
 20 25 30  
 15 Ala Ile Ala Pro Val Ala Ala His Ile Pro Leu Ala Ser Ala Ser Gly  
 35 40 45  
 Ser Gly Ser Gly Arg Ser Gly Ser Arg Ser Val Gly Ala Ser Thr Ser  
 20 50 55 60  
 Thr Ala Leu Ala Lys Ala Phe Asn Pro Phe Ser Glu Pro Ala Ser Phe  
 65 70 75 80  
 25 Ser Asp Ser Asp Lys Ser His Arg Ser Lys Thr Asn Lys Lys Pro Ser  
 85 90 95  
 Lys Ser Asp Ala Asn Arg Gln Phe Asn Glu Val His Lys Pro Arg Thr  
 100 105 110  
 30 Asp Gln Leu Glu Asn Ser Lys Asn Lys Ser Lys Gln Leu Val Asn Lys Pro  
 115 120 125  
 Asn His Asn Lys Met Ala Val Lys Glu Gln Arg Ser His His Lys Lys  
 130 135 140 145  
 Ser His His His Arg Ser His Gln Pro Lys Gln Ala Ser Ala Ser Thr  
 150 155 160  
 40 Glu Ser His Gln Ser Ser Ser Ile Glu Ser Ile Phe Val Glu Glu Pro  
 165 170 175  
 Thr Leu Val Leu Asp Arg Glu Val Ala Ser Ile Asn Val Pro Ala Ser  
 180 185 190  
 45 Ala Lys Ala Ile Ile Ala Glu Gln Gly Pro Ser Thr Tyr Ser Lys Glu  
 195 200 205  
 Ala Leu Ile Lys Asp Lys Leu Lys Pro Asp Pro Ser Thr Leu Val Glu  
 50 210 215 220 225

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	Ile	Glu	Lys	Ser	Leu	Leu	Ser	Leu	Phe	Asn	Met	Lys	Arg	Pro	Pro	Lys	
					230					235					240		
5	Ile	Asp	Arg	Ser	Lys	Ile	Ile	Ile	Pro	Glu	Pro	Met	Lys	Lys	Leu	Tyr	
				245					250					255			
	Ala	Glu	Ile	Met	Gly	His	Glu	Leu	Asp	Ser	Val	Asn	Ile	Pro	Lys	Pro	
				260				265					270				
10	Gly	Leu	Leu	Thr	Lys	Ser	Ala	Asn	Thr	Val	Arg	Ser	Phe	Thr	His	Lys	
		275					280					285					
	Asp	Ser	Lys	Ile	Asp	Asp	Arg	Phe	Pro	His	His	His	Arg	Phe	Arg	Leu	
	290					295					300					305	
15	His	Phe	Asp	Val	Lys	Ser	Ile	Pro	Ala	Asp	Glu	Lys	Leu	Lys	Ala	Ala	
					310					315					320		
	Glu	Leu	Gln	Leu	Thr	Arg	Asp	Ala	Leu	Ser	Gln	Gln	Val	Val	Ala	Ser	
20				325					330					335			
	Arg	Ser	Ser	Ala	Asn	Arg	Thr	Arg	Tyr	Gln	Val	Leu	Val	Tyr	Asp	Ile	
				340				345					350				
25	Thr	Arg	Val	Gly	Val	Arg	Gly	Gln	Arg	Glu	Pro	Ser	Tyr	Leu	Leu	Leu	
		355					360					365					
	Asp	Thr	Lys	Thr	Val	Arg	Leu	Asn	Ser	Thr	Asp	Thr	Val	Ser	Leu	Asp	
	370					375					380					385	
30	Val	Gln	Pro	Ala	Val	Asp	Arg	Trp	Leu	Ala	Ser	Pro	Gln	Arg	Asn	Tyr	
					390					395					400		
	Gly	Leu	Leu	Val	Glu	Val	Arg	Thr	Val	Arg	Ser	Leu	Lys	Pro	Ala	Pro	
35				405					410					415			
	His	His	His	Val	Arg	Leu	Arg	Arg	Ser	Ala	Asp	Glu	Ala	His	Glu	Arg	
				420				425					430				
40	Trp	Gln	His	Lys	Gln	Pro	Leu	Leu	Phe	Thr	Tyr	Thr	Asp	Asp	Gly	Arg	
		435					440					445					
	His	Lys	Ala	Arg	Ser	Ile	Arg	Asp	Val	Ser	Gly	Gly	Glu	Gly	Gly	Gly	
	450					455					460					465	
45	Lys	Gly	Gly	Arg	Asn	Lys	Arg	His	Ala	Arg	Arg	Pro	Thr	Arg	Arg	Lys	
					470					475					480		
	Asn	His	Asp	Asp	Thr	Cys	Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	
50					485				490					495			

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Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala  
 500 505 510  
 5 Tyr Tyr Cys His Gly Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn  
 515 520 525  
 Ser Thr Asn His Ala Val Val Gln Thr Leu Val Asn Asn Met Asn Pro  
 530 535 540 545  
 10 Gly Lys Val Pro Lys Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val  
 550 555 560  
 Ala Met Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr  
 565 570 575  
 15 Gln Glu Met Thr Val Val Gly Cys Gly Cys Arg  
 580 585

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 25 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..359  
 (D) OTHER INFORMATION: /note= "PRE-PRO-VG1"

## (x) PUBLICATION INFORMATION:

- (A) AUTHORS: WEEKS,  
 (C) JOURNAL: CELL  
 (D) VOLUME: 51  
 (F) PAGES: 861-867  
 40 (G) DATE: 1987

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Val Trp Leu Arg Leu Trp Ala Phe Leu His Ile Leu Ala Ile Val  
 1 5 10 15  
 Thr Leu Asp Pro Glu Leu Lys Arg Arg Glu Glu Leu Phe Leu Arg Ser  
 20 25 30  
 50 Leu Gly Phe Ser Ser Lys Pro Asn Pro Val Ser Pro Pro Pro Val Pro  
 35 40 45

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Ser Ile Leu Trp Arg Ile Phe Asn Gln Arg Met Gly Ser Ser Ile Gln  
 50 55 60  
 5 Lys Lys Lys Pro Asp Leu Cys Phe Val Glu Glu Phe Asn Val Pro Gly  
 65 70 75 80  
 Ser Val Ile Arg Val Phe Pro Asp Gln Gly Arg Phe Ile Ile Pro Tyr  
 85 90 95  
 10 Ser Asp Asp Ile His Pro Thr Gln Cys Leu Glu Lys Arg Leu Phe Phe  
 100 105 110  
 15 Asn Ile Ser Ala Ile Glu Lys Glu Glu Arg Val Thr Met Gly Ser Gly  
 115 120 125  
 Ile Glu Val Glu Pro Glu His Leu Leu Arg Lys Gly Ile Asp Leu Arg  
 130 135 140  
 20 Leu Tyr Arg Thr Leu Gln Ile Thr Leu Lys Gly Met  
 145 150 155  
 Gly Arg Ser Lys Thr Ser Arg Lys Leu Leu Val Ala Gln Thr Phe Arg  
 160 165 170  
 25 Leu Leu His Lys Ser Leu Phe Phe Asn Leu Thr Glu Ile Cys Gln Ser  
 180 185 190  
 30 Trp Gln Asp Pro Leu Lys Asn Leu Gly Leu Val Leu Glu Ile Phe Pro  
 195 200 205  
 Lys Lys Glu Ser Ser Trp Met Ser Thr Ala Asn Asp Glu Cys Lys Asp Ile  
 210 215 220 225  
 35 Gln Thr Phe Leu Tyr Thr Ser Leu Leu Thr Val Thr Leu Asn Pro Leu  
 230 235 240  
 Arg Cys Lys Arg Pro Arg Arg Lys Arg Ser Tyr Ser Lys Leu Pro Phe  
 245 250 255  
 40 Thr Ala Ser Asn Ile Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys  
 260 265 270  
 45 Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala  
 275 280 285 290  
 Asn Tyr Cys Tyr Gly Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn  
 295 300 305

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[illegible]

(2) INFORMATION FOR SEQ ID NO:14:

- ```

15      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 438 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

20      (ii) MOLECULE TYPE: protein

          (ix) FEATURE:
25          (A) NAME/KEY: Protein
          (B) LOCATION: 1..438
          (D) OTHER INFORMATION: /note= "PRE-PRO-VGR1"

          (x) PUBLICATION INFORMATION:
30          (A) AUTHORS: LYONS,
          (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
          (D) VOLUME: 86
          (F) PAGES: 4554-4558
          (G) DATE: 1989

```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Met | Arg | Lys | Met | Gln | Lys | Glu | Ile | Leu | Ser | Val | Leu | Gly | Pro | Pro | His |
|    | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |
| 40 |     | Arg | Pro | Arg | Pro | Leu | His | Gly | Leu | Gln | Pro | Gln | Pro | Pro | Val | Leu |
|    |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
|    | Pro | Pro | Gln | Gln | Gln | Gln | Gln | Gln | Gln | Gln | Gln | Gln | Thr | Ala | Asp | Glu |
| 45 |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
|    | Glu | Pro | Pro | Pro | Gly | Arg | Leu | Lys | Ser | Ala | Pro | Leu | Phe | Met | Leu | Asp |
|    | 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

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Leu Tyr Asn Ala Leu Ser Asn Asp Asp Glu Glu Asp Gly Ala Ser Glu  
 65 70 75 80  
 5 Gly Val Gly Gln Glu Pro Gly Ser His Gly Gly Ala Ser Ser Ser Gln  
 85 90 95  
 Leu Arg Gln Pro Ser Pro Gly Ala Ala His Ser Leu Asn Arg Lys Ser  
 100 105 110  
 10 Leu Leu Ala Pro Gly Pro Gly Gly Gly Ala Ser Pro Leu Thr Ser Ala  
 115 120 125  
 Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe Val  
 130 135 140  
 15 Asn Leu Val Gly Tyr Asp Lys Glu Phe Ser Pro His Gln Arg His His  
 145 150 155 160  
 Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile Pro Glu Gly Glu Ala Val  
 165 170 175  
 20 Thr Ala Ala Glu Phe Arg Val Tyr Lys Asp Cys Val Val Gly Ser Phe  
 180 185 190  
 25 Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu Ala  
 195 200 205  
 Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp  
 210 215 220  
 30 Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn  
 225 230 235 240  
 35 Leu Trp Val Val Ile Pro Gln His Asn Met Gly Leu Gln Leu Ser Val  
 245 250 255  
 Val Thr Arg Asp Gly Leu His Val Asn Pro Arg Ala Ala Gly Leu Val  
 260 265 270  
 40 Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe  
 275 280 285  
 Lys Val Ser Glu Val His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg  
 290 295 300  
 45 Arg Arg Gln Gln Ser Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ser  
 305 310 315 320  
 50 Arg Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr Ala  
 325 330 335



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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Met | Pro | Pro | Pro | Gln | Gln | Gly | Pro | Cys | Gly | His | His | Leu | Leu | Leu | Leu |  |
|    | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| 5  | Leu | Ala | Leu | Leu | Leu | Pro | Ser | Leu | Pro | Leu | Thr | Arg | Ala | Pro | Val | Pro |  |
|    |     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |
|    | Pro | Gly | Pro | Ala | Ala | Ala | Leu | Leu | Gln | Ala | Leu | Gly | Leu | Arg | Asp | Glu |  |
|    |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| 10 | Pro | Gln | Gly | Ala | Pro | Arg | Leu | Arg | Pro | Val | Pro | Pro | Val | Met | Trp | Arg |  |
|    |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
|    | Leu | Phe | Arg | Arg | Arg | Asp | Pro | Gln | Glu | Thr | Arg | Ser | Gly | Ser | Arg | Arg |  |
| 15 |     | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |
|    | Thr | Ser | Pro | Gly | Val | Thr | Leu | Gln | Pro | Cys | His | Val | Glu | Glu | Leu | Gly |  |
|    |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |
| 20 | Val | Ala | Gly | Asn | Ile | Val | Arg | His | Ile | Pro | Asp | Arg | Gly | Ala | Pro | Thr |  |
|    |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
|    | Arg | Ala | Ser | Glu | Pro | Val | Ser | Ala | Ala | Gly | His | Cys | Pro | Glu | Trp | Thr |  |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |
| 25 | Val | Val | Phe | Asp | Leu | Ser | Ala | Val | Glu | Pro | Ala | Glu | Arg | Pro | Ser | Arg |  |
|    |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
|    | Ala | Arg | Leu | Glu | Leu | Arg | Phe | Ala | Ala | Ala | Ala | Ala | Ala | Ala | Pro | Glu |  |
| 30 |     | 145 |     |     |     | 150 |     |     |     | 155 |     |     |     |     |     | 160 |  |
|    | Gly | Gly | Trp | Glu | Leu | Ser | Val | Ala | Gln | Ala | Gly | Gln | Gly | Ala | Gly | Ala |  |
|    |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |
| 35 | Asp | Pro | Gly | Pro | Val | Leu | Leu | Arg | Gln | Leu | Val | Pro | Ala | Leu | Gly | Pro |  |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |
|    | Pro | Val | Arg | Ala | Glu | Leu | Leu | Gly | Ala | Ala | Trp | Ala | Arg | Asn | Ala | Ser |  |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |
| 40 | Trp | Pro | Arg | Ser | Leu | Arg | Leu | Ala | Leu | Ala | Leu | Arg | Pro | Arg | Ala | Pro |  |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |
|    | Ala | Ala | Cys | Ala | Arg | Leu | Ala | Glu | Ala | Ser | Leu | Leu | Leu | Val | Thr | Leu |  |
| 45 |     | 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
|    | Asp | Pro | Arg | Leu | Cys | His | Pro | Leu | Ala | Arg | Pro | Arg | Arg | Asp | Ala | Glu |  |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |
| 50 | Pro | Val | Leu | Gly | Gly | Gly | Pro | Gly | Gly | Ala | Cys | Arg | Ala | Arg | Arg | Leu |  |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |

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Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro  
                   275                                  280                                  285  
 5 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val  
                   290                                  295                                  300  
 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu  
                   305                                  310                                  315                                  320  
 10 Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys  
                                   325                                  330                                  335  
 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn  
                                   340                                  345                                  350  
 15 Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu  
                   355                                  360                                  365  
 20 Cys Gly Cys Arg  
                   370

## (2) INFORMATION FOR SEQ ID NO:16:

- 25 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 455 amino acids  
     (B) TYPE: amino acid  
     (C) STRANDEDNESS: single  
     (D) TOPOLOGY: linear  
 30 (ii) MOLECULE TYPE: protein  
  
 (ix) FEATURE:  
 35 (A) NAME/KEY: Protein  
     (B) LOCATION: 1..455  
     (D) OTHER INFORMATION: /note= "PRE-PRO 60A"  
  
 (x) PUBLICATION INFORMATION:  
 40 (A) AUTHORS: WHARTON,  
     (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.  
     (D) VOLUME: 88  
     (F) PAGES: 9214-9218  
     (G) DATE: 1991  
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser  
 1                                  5                                  10                                  15

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|    |         |         |         |         |         |         |         |         |
|----|---------|---------|---------|---------|---------|---------|---------|---------|
|    | Leu Gly | Leu Gly | Met Val | Leu Leu | Met Phe | Val Ala | Thr Thr | Pro Pro |
|    |         | 20      |         |         | 25      |         | 30      |         |
| 5  | Ala Val | Glu Ala | Thr Gln | Ser Gly | Ile Tyr | Ile Asp | Asn Gly | Lys Asp |
|    |         | 35      |         | 40      |         | 45      |         |         |
|    | Gln Thr | Ile Met | His Arg | Val Leu | Ser Glu | Asp Asp | Lys Leu | Asp Val |
|    | 50      |         | 55      |         |         | 60      |         |         |
| 10 | Ser Tyr | Glu Ile | Leu Glu | Phe Leu | Gly Ile | Ala Glu | Arg Pro | Thr His |
|    | 65      |         | 70      |         | 75      |         |         | 80      |
|    | Leu Ser | Ser His | Gln Leu | Ser Leu | Arg Lys | Ser Ala | Pro Lys | Phe Leu |
|    |         | 85      |         |         | 90      |         |         | 95      |
| 15 | Leu Asp | Val Tyr | His Arg | Ile Thr | Ala Glu | Glu Gly | Leu Ser | Asp Gln |
|    |         | 100     |         | 105     |         |         | 110     |         |
| 20 | Asp Glu | Asp Asp | Tyr Glu | Arg Gly | His Arg | Ser Arg | Arg Ser | Ala     |
|    |         | 115     |         | 120     |         | 125     |         |         |
|    | Asp Leu | Glu Glu | Asp Glu | Gly Glu | Gln Gln | Lys Asn | Phe Ile | Thr Asp |
|    | 130     |         | 135     |         |         | 140     |         |         |
| 25 | Leu Asp | Lys Arg | Ala Ile | Asp Glu | Ser Asp | Ile Ile | Met Thr | Phe Leu |
|    | 145     |         | 150     |         |         | 155     |         | 160     |
|    | Asn Lys | Arg His | His Asn | Val Asp | Glu Leu | Arg His | Glu His | Gly Arg |
|    |         | 165     |         |         | 170     |         |         | 175     |
| 30 | Arg Leu | Trp Phe | Asp Val | Ser Asn | Val Pro | Asn Asp | Asn Tyr | Leu Val |
|    |         | 180     |         | 185     |         |         | 190     |         |
| 35 | Met Ala | Glu Leu | Arg Ile | Tyr Gln | Asn Ala | Asn Glu | Gly Lys | Trp Leu |
|    |         | 195     |         | 200     |         |         | 205     |         |
|    | Thr Ala | Asn Arg | Glu Phe | Thr Ile | Thr Val | Tyr Ala | Ile Gly | Thr Gly |
|    | 210     |         | 215     |         |         | 220     |         |         |
| 40 | Thr Leu | Gly Gln | His Thr | Met Glu | Pro Leu | Ser Ser | Val Asn | Thr Thr |
|    | 225     |         | 230     |         |         | 235     |         | 240     |
|    | Gly Asp | Tyr Val | Gly Trp | Leu Glu | Leu Asn | Val Thr | Glu Gly | Leu His |
|    |         | 245     |         |         | 250     |         | 255     |         |
| 45 | Glu Trp | Leu Val | Lys Ser | Lys Asp | Asn His | Gly Ile | Tyr Ile | Gly Ala |
|    |         | 260     |         | 265     |         |         | 270     |         |
| 50 | His Ala | Val Asn | Arg Pro | Asp Arg | Glu Val | Lys Leu | Asp Asp | Ile Gly |
|    |         | 275     |         | 280     |         |         | 285     |         |

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Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly  
 290 295 300  
 5 Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His  
 305 310 315 320  
 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser  
 325 330 335  
 10 Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg  
 340 345 350  
 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp  
 355 360 365  
 15 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser  
 370 375 380  
 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
 385 390 395 400  
 20 Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys-Lys Val Pro  
 405 410 415  
 25 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr  
 420 425 430  
 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile  
 435 440 445  
 30 Val Lys Ser Cys Gly Cys His  
 450 455

## (2) INFORMATION FOR SEQ ID NO:17:

- 35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 472 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 40 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein  
 45 (ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..472  
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP3"

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## (x) PUBLICATION INFORMATION:

(A) AUTHORS: WOZNEY,

(C) JOURNAL: SCIENCE

(D) VOLUME: 242

(F) PAGES: 1528-1534

(G) DATE: 1988

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 10 | Met | Ala | Gly | Ala | Ser | Arg | Leu | Leu | Phe | Leu | Trp | Leu | Gly | Cys | Phe | Cys |
|    | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
|    | Val | Ser | Leu | Ala | Gln | Gly | Glu | Arg | Pro | Lys | Pro | Pro | Phe | Pro | Glu | Leu |
|    |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| 15 | Arg | Lys | Ala | Val | Pro | Gly | Asp | Arg | Thr | Ala | Gly | Gly | Gly | Pro | Asp | Ser |
|    |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
|    | Glu | Leu | Gln | Pro | Gln | Asp | Lys | Val | Ser | Glu | His | Met | Leu | Arg | Leu | Tyr |
| 20 |     | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |
|    | Asp | Arg | Tyr | Ser | Thr | Val | Gln | Ala | Ala | Arg | Thr | Pro | Gly | Ser | Leu | Glu |
|    | 65  |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     |     | 80  |
| 25 | Gly | Gly | Ser | Gln | Pro | Trp | Arg | Pro | Arg | Leu | Leu | Arg | Glu | Gly | Asn | Thr |
|    |     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
|    | Val | Arg | Ser | Phe | Arg | Ala | Ala | Ala | Ala | Glu | Thr | Leu | Glu | Arg | Lys | Gly |
|    |     |     |     | 100 |     |     |     |     |     |     | 105 |     |     |     |     | 110 |
| 30 | Tyr | Ile | Phe | Asn | Leu | Thr | Ser | Leu | Thr | Lys | Ser | Glu | Asn | Ile | Leu | Ser |
|    |     |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |
|    | Ala | Thr | Leu | Tyr | Phe | Cys | Ile | Gly | Glu | Leu | Gly | Asn | Ile | Ser | Leu | Ser |
| 35 |     |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |
|    | Cys | Pro | Val | Ser | Gly | Gly | Cys | Ser | His | His | Ala | Gln | Arg | Lys | His | Ile |
|    |     | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     |
| 40 | Gln | Ile | Asp | Leu | Ser | Ala | Trp | Thr | Leu | Lys | Phe | Ser | Arg | Asn | Gln | Ser |
|    | 160 |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |
|    | Gln | Leu | Leu | Gly | His | Leu | Ser | Val | Asp | Met | Ala | Lys | Ser | His | Arg | Asp |
|    |     |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |
| 45 | Ile | Met | Ser | Trp | Leu | Ser | Lys | Asp | Ile | Thr | Gln | Phe | Leu | Arg | Lys | Ala |
|    |     |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |
|    | Lys | Glu | Asn | Glu | Glu | Phe | Leu | Ile | Gly | Phe | Asn | Ile | Thr | Ser | Lys | Gly |
| 50 |     |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |



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## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 453 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..453  
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP5 (HUMAN)"

## (x) PUBLICATION INFORMATION:

- (A) AUTHORS: CELESTE,  
 (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.  
 (D) VOLUME: 87  
 (F) PAGES: 9843-9847  
 (G) DATE: 1991

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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Met His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp
 1             5             10             15

Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn
 20             25             30

His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg
 35             40             45

Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
 50             55             60

Pro Arg Pro Phe Ser Pro Gly Lys Gln Ala Ser Ser Ala Pro Leu Phe
 65             70             75             80

Met Leu Asp Leu Tyr Asn Ala Met Thr Asn Glu Glu Asn Pro Glu Glu
 85             90             95

Ser Glu Tyr Ser Val Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala
100            105            110

Arg Lys Gly Tyr Pro Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile
115            120            125

```

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|    |     |     |     |     |     |     |     |     |     |     |     |     |         |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|-----|-----|-----|
|    | Gln | Leu | Ser | Arg | Thr | Thr | Pro | Leu | Thr | Thr | Gln | Ser | Pro     | Pro | Leu | Ala |
|    |     | 130 |     |     |     |     |     | 135 |     |     |     |     | 140     |     |     |     |
| 5  | Ser | Leu | His | Asp | Thr | Asn | Phe | Leu | Asn | Asp | Ala | Asp | Met     | Val | Met | Ser |
|    |     | 145 |     |     |     |     | 150 |     |     |     |     | 155 |         |     |     |     |
|    | Phe | Val | Asn | Leu | Val | Glu | Arg | Asp | Lys | Asp | Phe | Ser | His     | Gln | Arg | Arg |
|    | 160 |     |     |     |     | 165 |     |     |     |     | 170 |     |         |     | 175 |     |
| 10 | His | Tyr | Lys | Glu | Arg | Phe | Asp | Leu | Thr | Gln | Ile | Pro | His     | Gly | Glu | Ala |
|    |     |     |     |     | 180 |     |     |     |     | 185 |     |     |         |     | 190 | Val |
|    | Thr | Ala | Ala | Glu | Phe | Arg | Ile | Val | Lys | Asp | Arg | Ser | Asn     | Asn | Arg | Phe |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205     |     |     |     |
| 15 | Glu | Asn | Glu | Thr | Ile | Lys | Ile | Ser | Ile | Tyr | Gln | Ile | Ile     | Lys | Glu | Tyr |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |         |     |     |     |
|    | Thr | Asn | Arg | Asp | Ala | Asp | Leu | Phe | Leu | Leu | Asp | Thr | Arg     | Lys | Ala | Gln |
| 20 | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |         |     |     | 240 |
|    | Ala | Leu | Asp | Val | Gly | Trp | Leu | Val | Phe | Asp | Ile | Thr | Val-Thr | Ser | Asn |     |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |         | 255 |     |     |
| 25 | His | Trp | Val | Ile | Asn | Pro | Gln | Asn | Asn | Leu | Gly | Leu | Gln     | Leu | Cys | Ala |
|    |     |     |     |     | 260 |     |     |     | 265 |     |     |     |         | 270 |     |     |
|    | Glu | Thr | Gly | Asp | Gly | Arg | Ser | Ile | Asn | Val | Lys | Ser | Ala     | Gly | Leu | Val |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285     |     |     |     |
| 30 | Gly | Arg | Gln | Gly | Pro | Gln | Ser | Lys | Gln | Pro | Phe | Met | Val     | Ala | Phe | Phe |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |         |     |     |     |
|    | Lys | Ala | Ser | Glu | Val | Leu | Leu | Arg | Ser | Val | Arg | Ala | Ala     | Asn | Lys | Arg |
| 35 | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |         |     |     | 320 |
|    | Lys | Asn | Gln | Asn | Arg | Asn | Lys | Ser | Ser | Ser | His | Gln | Asp     | Ser | Ser | Arg |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |         |     | 335 |     |
| 40 | Met | Ser | Ser | Val | Gly | Asp | Tyr | Asn | Thr | Ser | Glu | Gln | Lys     | Gln | Ala | Cys |
|    |     |     |     | 340 |     |     |     |     | 345 |     |     |     |         | 350 |     |     |
|    | Lys | Lys | His | Glu | Leu | Tyr | Val | Ser | Phe | Arg | Asp | Leu | Gly     | Trp | Gln | Asp |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365     |     |     |     |
| 45 | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala | Ala | Phe | Tyr | Cys     | Asp | Gly | Glu |
|    |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |         |     |     |     |
|    | Cys | Ser | Phe | Pro | Leu | Asn | Ala | His | Met | Asn | Ala | Thr | Asn     | His | Ala | Ile |
| 50 | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |         |     |     | 400 |

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Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys Pro  
 405 410 415

5 Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp  
 420 425 430

Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg  
 435 440 445

10 Ser Cys Gly Cys His  
 450

## (2) INFORMATION FOR SEQ ID NO:19:

- 15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 513 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- (ix) FEATURE:  
 25 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..513  
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP6 (HUMAN)"
- (x) PUBLICATION INFORMATION:  
 30 (A) AUTHORS: CELESTE,  
 (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.  
 (D) VOLUME: 87  
 (F) PAGES: 9843-9847  
 (G) DATE: 1991

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly  
 1 5 10 15

40 Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro  
 20 25 30

45 Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly  
 35 40 45

Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser  
 50 55 60

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|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Gly | Phe | Leu | Tyr | Arg | Arg | Leu | Lys | Thr | Gln | Glu | Lys | Arg | Glu | Met | Gln |
|    | 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| 5  | Lys | Glu | Ile | Leu | Ser | Val | Leu | Gly | Leu | Pro | His | Arg | Pro | Arg | Pro | Leu |
|    |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
|    | His | Gly | Leu | Gln | Gln | Pro | Gln | Pro | Pro | Ala | Leu | Arg | Gln | Gln | Glu | Glu |
|    |     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| 10 | Gln | Gln | Gln | Gln | Gln | Gln | Leu | Pro | Arg | Gly | Glu | Pro | Pro | Pro | Gly | Arg |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
|    | Leu | Lys | Ser | Ala | Pro | Leu | Phe | Met | Leu | Asp | Leu | Tyr | Asn | Ala | Leu | Ser |
| 15 |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
|    | Ala | Asp | Asn | Asp | Glu | Asp | Gly | Ala | Ser | Glu | Gly | Glu | Arg | Gln | Gln | Ser |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
|    | Trp | Pro | His | Glu | Ala | Ala | Ser | Ser | Ser | Gln | Arg | Arg | Gln | Pro | Pro | Pro |
| 20 |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
|    | Gly | Ala | Ala | His | Pro | Leu | Asn | Arg | Lys | Ser | Leu | Leu | Ala | Pro | Gly | Ser |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| 25 | Gly | Ser | Gly | Gly | Ala | Ser | Pro | Leu | Thr | Ser | Ala | Gln | Asp | Ser | Ala | Phe |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
|    | Leu | Asn | Asp | Ala | Asp | Met | Val | Met | Ser | Phe | Val | Asn | Leu | Val | Glu | Tyr |
| 30 |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
|    | Asp | Lys | Glu | Phe | Ser | Pro | Arg | Gln | Arg | His | His | Lys | Glu | Phe | Lys | Phe |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
|    | Asn | Leu | Ser | Gln | Ile | Pro | Glu | Gly | Glu | Val | Val | Thr | Ala | Ala | Glu | Phe |
| 35 |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
|    | Arg | Ile | Val | Lys | Asp | Cys | Val | Met | Gly | Ser | Phe | Lys | Asn | Gln | Thr | Phe |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| 40 | Leu | Ile | Ser | Ile | Tyr | Gln | Val | Leu | Gln | Glu | His | Gln | His | Arg | Asp | Ser |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
|    | Asp | Leu | Phe | Leu | Leu | Asp | Thr | Arg | Val | Val | Trp | Ala | Ser | Glu | Glu | Gly |
|    | 290 |     |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| 45 | Trp | Leu | Glu | Phe | Asp | Ile | Thr | Ala | Thr | Ser | Asn | Leu | Trp | Val | Val | Thr |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |

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[illegible]

40 (2) INFORMATION FOR SEQ ID NO:20:

**(i) SEQUENCE CHARACTERISTICS:**

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

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## (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= Generic-Seq-7

5 /note= "wherein each Xaa is independently selected  
from a group of one or more specified amino acids  
as defined in the specification."

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15

Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro  
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa  
35 40 45

20 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro  
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa  
25 65 70 75 80

Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys  
85 90 95

30 Xaa

## (2) INFORMATION FOR SEQ ID NO:21:

## 35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: protein

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## (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= Generic-Seq-8

/note= "wherein each Xaa is independently selected  
from a group of one or more specified amino acids  
as defined in the specification."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa  
1 5 10 15  
Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly  
20 25 30  
Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala  
35 40 45  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
50 55 60  
Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa  
65 70 75 80  
Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val  
85 90 95  
Xaa Xaa Cys Xaa Cys Xaa  
100

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS  
AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

5 Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa  
 1 5 10 15  
 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly  
 20 25 30  
 10 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys  
 50 55 60  
 15 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa  
 65 70 75 80  
 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val  
 85 90 95  
 20 Xaa Ala Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:23:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 4 amino acids  
 (B) TYPE: amino acid  
 30 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: peptide  
 35 (ix) FEATURE:  
 (A) NAME/KEY: Cleavage-site  
 (B) LOCATION: 1..4  
 (D) OTHER INFORMATION: /note= "PROTEOLYTIC CLEAVAGE SITE"

40

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Xaa Xaa Arg  
 1

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What is claimed is:

1. Dimeric protein comprising a pair of protein subunits associated to defined a dimeric structure having morphogenic activity,  
each of said subunits comprising at least a 100 amino acid sequence having a pattern of cysteine residues characteristic of the morphogen family,  
at least one of said subunits comprising a mature form of a subunit of a member of the morphogen family, or an allelic, species, or sequence variant thereof, noncovalently complexed with  
a peptide comprising a pro region of a member of the morphogen family, or an allelic, species, or sequence variant thereof to form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.
2. The protein of claim 1 wherein both said subunits comprise a mature form of a subunit of a member of the morphogen family or an allelic, species, or sequence variant thereof, each said subunit being noncovalently complexed with a said peptide.
3. The protein of claim 1 wherein each said subunit is the mature form of human OP-1, or a species or allelic variant thereof.
4. The protein of claim 1, 2, or 3 wherein the peptide comprises the pro region of human OP-1, or a species, allelic or sequence variant thereof.

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5. The protein of claim 1 wherein said peptide comprises at least the first 18 amino acids of an amino acid sequence defining said pro region.

6. The protein of claim 1 wherein said peptide comprises at least the first 18 amino acids of an amino acid sequence defining said pro region in Seq. ID Nos. 1-16 or a sequence variant thereof.

7. The protein of claim 1 or 6 wherein said peptide comprises the full length form of said pro region.

8. The protein of claim 1 wherein said pro region peptide comprises an amino acid sequence selected from sequences defined by residues 30-48, 30-292 and 48-292 of Seq. ID No. 1.

9. The protein of claim 1 wherein said pro region peptide comprises an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA encoding the N-terminal 18 amino acids of the pro region sequences for Seq. ID Nos. 1-19.

10. The protein of claims 1 or 9 wherein said pro region peptide comprises a nucleic acid that hybridizes under stringent conditions with a DNA defined by nucleotides of 136-192 of Seq. ID No. 1 or nucleotides 157-211 of Seq. ID No. 5.

11. The protein of claim 1 wherein said subunit sequence variant comprises a chimeric morphogen amino acid sequence.

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12. The protein of claim 1 wherein said peptide comprises a chimeric pro region amino acid sequence.
13. The protein of claim 1 wherein said subunit comprises a sequence defined by Generic Sequence 7 or Generic Sequence 8.
14. The protein of claim 1 wherein said subunit comprises a sequence having 60% amino acid identity with the sequence defined by residues 335-431 of Seq. ID No.1.
15. The protein of claim 1 wherein said subunit comprises the mature form of a subunit defined by any of the sequences of Seq. ID No. 5-19.
16. The protein of claim 1 wherein said subunit comprises an amino acid sequence encoded by a nucleic acid that hybridizes with a DNA defined by nucleotides 1036-1341 of Seq. ID No. 1 or nucleotides 1390-1695 of Seq. ID No. 5.
17. The protein of claim 1 further comprising an molecule capable of enhancing the stability of said complex.
18. A therapeutic composition comprising the protein of any of claims 1, 2, 5-9 or 11-17.
19. A therapeutic composition comprising the protein of claim 1 wherein each said subunit is the mature form of human OP-1, or a species or allelic variant thereof.

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20. A therapeutic composition comprising the protein of claim 1, wherein said peptide comprises part or all of the pro region of human OP-1, or a species or allelic variant thereof.

21. The therapeutic composition of claim 18 comprising the protein of claim 1 wherein said subunit comprises the mature form of a subunit defined by any of the sequences of Seq. ID Nos. 5-19.

22. A therapeutic composition comprising the protein of claims 3, 4 or 10.

23. The therapeutic composition of claims 18 or 22 further comprising a cofactor.

24. The therapeutic composition of claim 23 wherein said cofactor is a symptom-alleviating cofactor.

25. A kit for diagnosing a tissue disorder or evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the kit comprising:

- a) means for capturing a cell or fluid sample from said mammal,
- b) a binding protein capable of interacting specifically with a soluble morphogen complex in said sample, and
- c) means for detecting the binding protein bound to said soluble morphogen complex.

26. The kit of claim 25 wherein said binding protein is an antibody.

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27. A method for evaluating the status of a tissue, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

28. A method for evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

29. A method for diagnosing a tissue disorder in a mammal, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

30. The invention of claim 25, 26, 27 or 28 wherein said morphogen is a dimeric protein comprising a pair of protein subunits associated to defined a dimeric structure having morphogenic activity,

each of said subunits comprising at least a 100 amino acid sequence having a pattern of cysteine residues characteristic of the morphogen family,

at least one of said subunits comprising a mature form of a subunit of a member of the morphogen family, or an allelic, species, or sequence variant thereof, noncovalently complexed with

a peptide comprising a pro region of a member of the morphogen family, or an allelic, species, or sequence variant thereof to form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.

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31. The invention of claims 25, 26, 27 or 28 wherein said quantity of morphogen is detected by an immunoassay.

32. The invention of claims 25, 26, 27 or 28 wherein said quantity of morphogen is detected by an antibody capable of distinguishing soluble morphogen in a sample fluid.

33. The invention of claims 25, 26, 27 or 28 wherein said body fluid sample comprises serum.

34. The invention of claims 25 or 28 wherein said tissue disorder is a bone tissue disorder.

35. The invention of claim 34 wherein said bone tissue disorder is selected from the group consisting of osteosarcoma, osteoporosis, and Paget's disease.

36. A method of evaluating the status of a tissue, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

37. A method for evaluating the efficacy of a therapy to regenerate lost or damaged tissue, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

38. A method for diagnosing a tissue disorder, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

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39. A kit for diagnosing a tissue disorder or evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the kit comprising:

a) means for capturing a cell or fluid sample from said mammal;

b) a binding protein capable of interacting specifically with an endogenous anti-morphogen antibody in said sample; and

c) means for detecting said binding protein-bound to said endogenous anti-morphogen antibody.

1/2

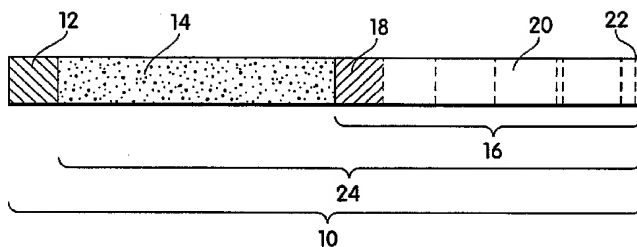


Fig. 1

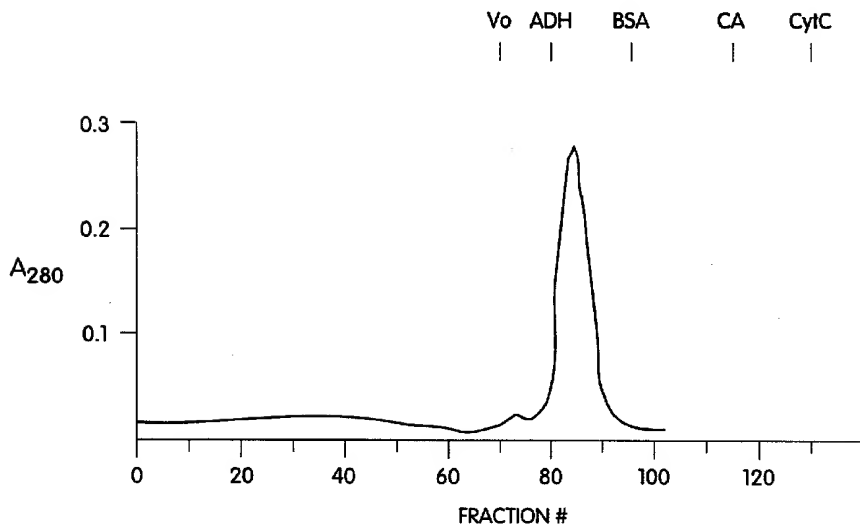


Fig. 3

2/2

OP-2: RAPRQQPFVVTFFRASPSPIRTPRAVRPLRRRQPKKSNELPQANRLPGIFDDVHGSHGRQVC  
 OP-1: RSIRSTGSKORSQNRSKTPKNQ~~EAL~~RMANVAENSSSDQRQAC  
 Vgr-1: RTTRSASSRRRQQSRNRSTQSQDVSRGSGSSDYNGSELKTAC  
 BMP-5: RSVRAANKRKNQNRNKSSSHQDSSRMSSVGDYNTSEQKQAC  
 60A: RSKRSASHPRKRKKSVSPNNVPLLEPMESTRSC  
 DPP: RSIRDVSGEGGGKGRNKRHARRPTRRKNHDDTC  
 BMP-2: RHVRISRSLHODEHSWSQIRPLLVTFGHDGKGPHLK--REKRQAKH--KQRRLKSSC  
 BMP-4: RISRSLPQSGGNWAQLRPLLVTFGHDGRGHALTRRRRAKRSPKHHSQARAKKNKNC  
 Vg-1: RCKRPRRRRSYSKLPPTASNIC  
 BMP-3: RKKRSTGVLLPLQ.....KSKNKKQKRGPHRKSTLQFDEQTLKKARRKQWIEPRNC

Fig. 2

## INTERNATIONAL SEARCH REPORT

 Int. Application No  
 PCT/US 93/07189

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/12 A61K37/02 G01N33/50 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                  | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | WO,A,91 18047 (GENENTECH, INC.) 28<br>November 1991<br>see page 2, line 24 - page 3, line 4<br>see page 4, line 4 - line 8<br>see page 5, line 16 - page 6, line 5<br>---                                                                                                                                                                                                                           | 1,5,7,12              |
| X          | MOLECULAR ENDOCRINOLOGY<br>vol. 5, no. 1, January 1991<br>pages 149 - 155<br>R. GLENN HAMMONDS, JR. ET AL.<br>'Bone-inducing activity of mature BMP-2b<br>produced from a hybrid BMP-2a/2b<br>precursor'<br>see abstract<br>see page 149, right column, paragraph 3 -<br>page 150, left column, paragraph 3<br>see page 152, left column, paragraph 2 -<br>right column, paragraph 3<br>---<br>-/-- | 1,5,7,12              |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

2 November 1993

Date of mailing of the international search report

14. 12. 93

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Authorized officer

MONTERO LOPEZ, B

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/07189

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                |                       |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category *                                           | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                             | Relevant to claim No. |
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